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[Continued on next page]

(54) Title: IMPROVED INTERFERON THERAPY

(57) Abstract: Interferon therapy is improved by concomitant administration of an agent which minimizes the ability of interferon to up-regulate expression of Programmed Cell Death Protein 1 (also known as CD279).

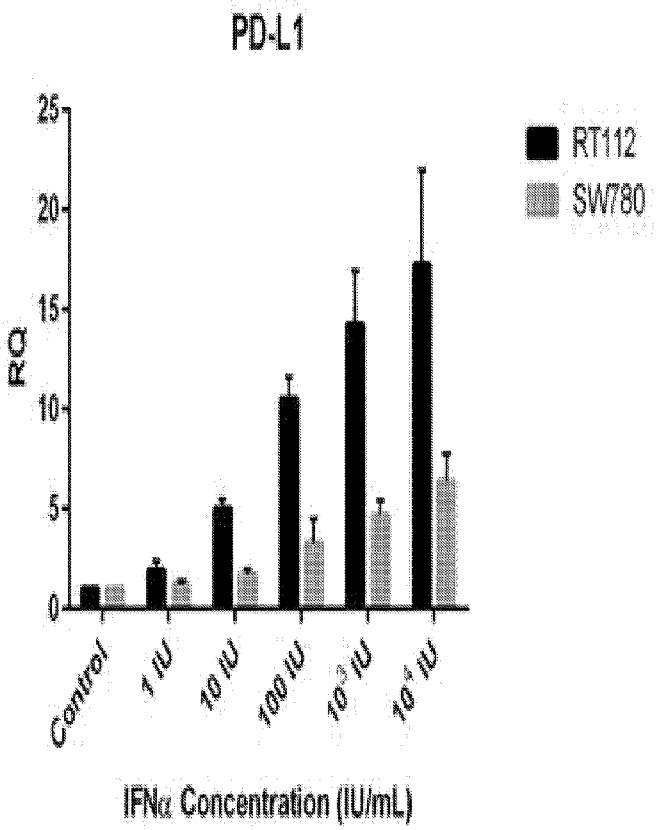

Figure 1

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— *as to the identity of the inventor (Rule 4.17(i))*

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

— *of inventorship (Rule 4.17(iv))*

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Improved Interferon Therapy

Applicant: FKD Therapies Limited, Chinnor, Oxfordshire England, citizen of the United Kingdom.

Related Applications: This application asserts priority from provisional patent filing serial no US62/295268, filed 15 February 2016, the contents of which are here incorporated by reference.

Federally-Sponsored Research & Development: None.

Joint Research Agreement: Applicant has research agreements with *inter alia* M.D. Anderson Cancer Center (Houston, Texas) and The Mayo Clinic (Rochester, Minnesota) for work related to this application.

Sequence Listing: None.

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Background:

Interferon has many clinical benefits. For example, interferon is known to up-regulate the immune system. It thus is potentially useful for recruiting the patient's innate immune system to identify and attack cancer cells. Interferon's efficacy as an anti-cancer agent, however, has to date proven wanting. This has been puzzling.

For example, the most effective bladder cancer treatment currently approved in The United States is intra-urethral *Bacillus Calmette-Guérin* vaccine. The antigenic vaccine is thought to stimulate bladder cells to express interferon, which in turn recruits the patient's innate immune system to better recognize cancer cell surface antigens and attack cancer cells. In over a third of cases, however, the vaccine is ineffective.

Similarly, intravesical instillation of exogenously manufactured interferon polypeptide has been tested to treat bladder cancer, but has been found less effective than expected.

Ihave discovered why, and figured out how to fix it.

Brief Description:

Ihave found that interferon (either exogenously administered or expressed in response to a vaccine or other agent which up-regulates endogenous expression), in addition to stimulating interferon expression, also stimulates the expression of Programmed Cell Death Protein 1, also known as CD279. Ihave thus identified a previously-unrecognized adverse side effect of interferon therapy: interferon advantageously stimulates certain aspects of the patient's immune system, yet also up-regulates expression of Programmed Cell Death Protein 1. The resulting increase in Programmed Cell Death Protein 1 in turn down-regulates protective T cell function. This impairs the effectiveness of T cells in identifying and attacking cells bearing cancer cell-surface antigen. Thus, interferon produces two conflicting actions: it both increases immune system activity, yet inhibits the ability of the immune system to identify cancer cell-surface antigens.

Ithus propose improving interferon therapy by co-administering an agent which inhibits the expression of Programmed Cell Death Protein 1. This will enable interferon to more fully achieve its therapeutic potential.

Brief Description of the Figures:

Figure 1 is a chart measuring PD-L1 expression in response to interferon exposure, for the RT112 and SW780 human cell lines. Horizontal axis: interferon amount. Vertical axis: polypeptide expressed.

Figure 2 is a chart measuring TRAIL expression in response to interferon exposure, for the RT112 and SW780 human cell lines. Horizontal axis: interferon amount. Vertical axis: polypeptide expressed.

Figure 3 is a chart measuring IRF1 expression in response to interferon exposure, for the RT112 and SW780 human cell lines. Horizontal axis: interferon amount. Vertical axis: polypeptide expressed.

Figure 4 is a photograph of a PAGE gel showing *in vitro* dose response to increasing interferon alpha, in an SW780 human cancer cell line. Horizontal axis: interferon amount. Vertical axis: polypeptide expressed.

Figure 5 measures expression in RT112 cells of IRF1, FOXA1 and PD-L1 in response to interferon exposure, *see* Example 2. IRF1 served as an interferon-stimulated gene control. FOXA1 is an example of a type I interferon regulated gene that did not change expression after interferon exposure.

Figure 6 measures expression in UC3 cells of IRF1, FOXA1 and PD-L1 in response to interferon exposure, *see* Example 2. IRF1 served as an interferon-stimulated gene control. FOXA1 is an example of a type I interferon regulated gene that did not change expression after interferon exposure.

Figure 7 measures expression in T24 cells of IRF1, FOXA1 and PD-L1 in response to interferon exposure, *see* Example 2. IRF1 served as an interferon-stimulated gene control. FOXA1 is an example of a type I interferon regulated gene that did not change expression after interferon exposure.

Figure 8 measures expression in UC14 cells of IRF1, FOXA1 and PD-L1 in response to interferon exposure, *see* Example 2. IRF1 served as an interferon-stimulated gene control. FOXA1 is an example of a type I interferon regulated gene that did not change expression after interferon exposure.

Figure 9 is a photograph of a 6-lane PAGE gel. It measures the presence of PD-L1 polypeptide after exposing BBN972 cells to murine interferon. Lanes are (left to right) 0

(zero), 1 x 10^0, 1 x 10^1, 1 x 10^2, 1 x 10^3 and 1 x 10^4 international units interferon / mL of culture medium.

Figure 10 is a photograph of a 6-lane PAGE gel. It measures the presence of PD-L1 polypeptide after exposing MB49 #1 (MB49-*luc*) cells to murine interferon. Lanes are (left to right) 0 (zero), 1 x 10^0, 1 x 10^1, 1 x 10^2, 1 x 10^3 and 1 x 10^4 international units interferon / mL of culture medium.

Figure 11 is a photograph of a 6-lane PAGE gel. It measures the presence of actin polypeptide after exposing BBN972 cells to murine interferon. Lanes are (left to right) 0 (zero), 1 x 10^0, 1 x 10^1, 1 x 10^2, 1 x 10^3 and 1 x 10^4 international units interferon / mL of culture medium.

Figure 12 is a photograph of a 6-lane PAGE gel. It measures the presence of actin polypeptide after exposing MB49 #1 cells to murine interferon. Lanes are (left to right) 0 (zero), 1 x 10^0, 1 x 10^1, 1 x 10^2, 1 x 10^3 and 1 x 10^4 international units interferon / mL of culture medium.

Figure 13 measures serum interferon a in mice in response to intra-peritoneal injection of Poly I:C.

Figure 14 measures serum interferon a in mice in response to intra-tumoral injection of Poly I:C at 6 hours.

Figure 15 measures PD-L1 expression intra-tumorally 24 hours after Poly I:C (500 mcg) intra-peritoneal injection.

Figure 16 shows RNA expression in humans treated with INSTILADRIN™ recombinant replication-deficient adenovirus gene therapy vector carrying a human interferon alpha 2B transgene.

Figure 17 shows MB49 tumor size vs time, for subcutaneous C57BL6/J tumors (n = 5 female mice per group). Treatment is 200 mcg q3 days starting on day 10 after tumor implant. Error bard represent SEM.

Figure 18 shows a Kaplan-Meyer survival curve for female mice with inoculated tumors, treated with saline (lowermost line), IgG (next higher line), anti-PD1 monoclonal antibody (next higher line), Poly I:C (next higher line) and a combination of Poly I:C and anti-PD1 monoclonal antibody (highest line).

Figure 19 compares normalized (mean +/- SD) radiance over time in male mice. Using a log-rank test, these data show combination therapy superior to IgG control ($p = 0.06$), superior to Poly I:C monotherapy ($p = 0.32$), and superior to anti-PD1 monoclonal antibody ($p = 0.14$).

Figure 20 shows "survival portions," *i.e.*, data showing the survival of propensity to survive over time, in male mice treated per Figure 19.

Detailed Description:

Interferon Therapy

Interferons are a group of signaling proteins. They are expressed and secreted by human cells in response to the presence of several antigenic pathogens, *e.g.*, viruses, bacteria and parasites, and also tumor cells. Typically, a virus-infected cell releases interferons, signaling nearby bystander cells to heighten their anti-viral defenses. Interferons also activate immune cells such as natural killer cells and macrophages. Interferons increase expression of major histocompatability complex antigens, which in turn increases presentation of foreign antigens to the immune system.

Interferons may be sorted or classified according to the type of receptor through which they signal. For humans, interferons are often thus sorted into three kinds: Type I

(interferons which bind to human IFN-α/β receptors), Type II (interferons which binds to the human IFN-γ receptor) and Type III (interferons which bind to human IFN-λ receptors).

All interferons share several common effects: they are antiviral agents and they modulate functions of the immune system. Administration of Type I IFN has been shown to inhibit tumor growth in experimental animals, but the beneficial action in human tumors has not been widely documented. A virus-infected cell releases viral particles that can infect nearby cells. However, the infected cell can prepare neighboring cells against a potential infection by the virus by releasing interferons. In response to interferon, cells produce large amounts of an enzyme known as protein kinase R (PKR). This enzyme phosphorylates a protein known as eIF-2 in response to new viral infections; the phosphorylated eIF-2 forms an inactive complex with another protein, called eIF2B, to reduce protein synthesis within the cell. Another cellular enzyme, RNAse L—also induced by interferon action—destroys RNA within the cells to further reduce protein synthesis of both viral and host genes. Inhibited protein synthesis destroys both the virus and infected host cells. In addition, interferons induce production of hundreds of other proteins—known collectively as interferon-stimulated genes (ISGs)—that have roles in combating viruses and other actions produced by interferon. They also limit viral spread by increasing p53 activity, which kills virus-infected cells by promoting apoptosis. The effect of IFN on p53 is also linked to its protective role against certain cancers.

Another function of interferons is to up-regulate expression of major histocompatability complex molecules, MHC I and MHC II, and increase immune-proteasome activity. Higher MHC I expression increases presentation of viral peptides to cytotoxic T cells, while the immune-proteasome processes viral peptides for loading onto the MHC I molecule, thereby increasing the recognition and killing of infected cells. Higher MHC II expression increases presentation of viral peptides to helper T cells; these cells

release cytokines (such as more interferons and interleukins, among others) that signal to and co-ordinate the activity of other immune cells.

Production of interferons occurs mainly in response to microbes, such as viruses and bacteria, and their products. Binding of molecules uniquely found in microbes—viral glycoprotein, viral RNA, bacterial endotoxin (lipopolysaccharide), bacterial flagella, CpG motifs—by pattern recognition receptors, such as membrane bound Toll like receptors or the cytoplasmic receptors RIG-I or MDA5, can trigger release of IFNs. Toll Like Receptor 3 (TLR3) is important for inducing interferons in response to the presence of double-stranded RNA viruses; the ligand for this receptor is double-stranded RNA (dsRNA). After binding dsRNA, this receptor activates the transcription factors IRF3 and NF-kB, which are important for initiating synthesis of many inflammatory proteins. RNA interference technology tools such as siRNA or vector-based reagents can either silence or stimulate interferon pathways. Release of IFN from cells (specifically IFN in lymphoid cells) is also induced by mitogens. Other cytokines, such as interleukin 1, interleukin 2, interleukin-12, tumor necrosis factor and colony-stimulating factor, can also enhance interferon production.

Interferon therapy is used (in combination with chemotherapy and radiation) as a treatment for some cancers. This treatment can be used in hematological malignancy; leukemia and lymphomas including hairy cell leukemia, chronic myeloid leukemia, nodular lymphoma, and cutaneous T-cell lymphoma. Patients with recurrent melanomas receive recombinant IFN-a2b. Both hepatitis B and hepatitis C are treated with IFN-b, often in combination with other antiviral drugs. Some of those treated with interferon have a sustained virological response and can eliminate hepatitis virus. The most harmful strain—hepatitis C genotype I virus—can be treated with a 60-80% success rate with the current standard-of-care treatment of interferon, RIBAVIRIN™ and recently approved protease inhibitors such as Telaprevir (Incivek™) May 2011, Boceprevir (VICTRELIS™) May 2011

171 or the nucleotide analog polymerase inhibitor Sofosbuvir (SOVALDI™) December 2013.
172 Biopsies of patients given the treatment show reductions in liver damage and cirrhosis. Some
173 evidence shows giving interferon immediately following infection can prevent chronic
174 hepatitis C, although diagnosis early in infection is difficult since physical symptoms are
175 sparse in early hepatitis C infection. Control of chronic hepatitis C by IFN is associated with
176 reduced hepato-cellular carcinoma.

177 The art teaches interferon may be administered as an exogenous polypeptide.

178 Alternatively, one may induce endogenous expression of native interferon genes. For
179 example, the art teaches *e.g.*, antigenic *Bacillus Calmette-Guérin* or *Mycobacterium* or
180 *Adenovirus* vaccines. Such antigenic preparations induce the patient's own cells to express
181 interferon.

182 Alternatively, one may induce endogenous expression of a non-native interferon
183 transgene by transfecting a host cell with a vector delivering the interferon transgene. Indeed,
184 even exogenously-administered interferon polypeptide itself acts as a messenger to stimulate
185 interferon production.

186 As used herein, the term "interferon" (abbreviated "IFN") refers collectively to type 1
187 and type 2 interferons including deletion, insertion, or substitution variants thereof,
188 biologically active fragments, and allelic forms. As used herein, the term interferon
189 (abbreviated "IFN") refers collectively to type 1 and type 2 interferons. Type 1 interferon
190 includes interferons-α, -β and -ω and their subtypes. Human interferon-α has at least 14
191 identified subtypes while interferon-β has 3 identified subtypes. Particularly, preferred
192 interferon-alphas include human interferon alpha subtypes including, but not limited to, α-1
193 (GenBank Accession Number NP 076918), α-1b (GenBank Accession Number AAL35223),
194 α-2, α-2a (GenBank Accession Number NP000596), α-2b (GenBank Accession Number
195 AAP20099), α-4 (GenBank Accession Number NP066546), α-4b (GenBank Accession

Number CAA26701), α-5 (GenBank Accession Numbers NP 002160 and CAA26702), α-6 (GenBank Accession Number CAA26704), α-7 (GenBank Accession Numbers NP 066401 and CAA 26706), α-8 (GenBank Accession Numbers NP002161 and CAA 26903), α-10 (GenBank Accession Number NP 002162), α-13 (GenBank Accession Numbers NP 008831 and CAA 53538), α-14 (GenBank Accession Numbers NP 002163 and CAA 26705), α-16 (GenBank Accession Numbers NP 002164 and CAA 26703), α-17 (GenBank Accession Number NP 067091), α-21 (GenBank Accession Numbers P01568 and NP002166), and consensus interferons as described in Stabinsky, U.S. Pat. No. 5,541,293, issued Jul. 30, 1996, Stabinsky, U.S. Pat. No. 4,897,471, issued Jan. 30, 1990, and Stabinsky, U.S. Pat. No. 4,695,629, issued Sep. 22, 1987, the teachings of which are herein incorporated by reference, and hybrid interferons as described in Goeddel et al., U.S. Pat. No. 4,414,150, issued Nov. 8, 1983, the teachings of which are herein incorporated by reference. Type 2 interferons are referred to as interferon γ (EP 77,670A and EP 146,354A) and subtypes. Human interferon gamma has at least 5 identified subtypes, including interferon omega 1 (GenBank Accession Number NP 002168). Construction of DNA sequences encoding inteferons for expression may be accomplished by conventional recombinant DNA techniques based on the well-known amino acid sequences referenced above and as described in Goeddel et al., U.S. Pat. No. 6,482,613, issued Nov. 19, 2002, the teachings of which are herein incorporated by reference.

"Biologically active" fragments of interferons may be identified as having any anti-tumor or anti-proliferative activity as measured by techniques well known in the art (see, for example, Openakker et al., supra; Mossman, *J. Immunol. Methods,* 65:55 (1983) and activate IFN responsive genes through IFN receptor mediated mechanisms. Soluble IFN-αand IFN-β proteins are generally identified as associating with the Type 1 IFN receptor complex (GenBank Accession Number NP 000865) and activate similar intracellular signaling

pathways. IFN-γ is generally identified as associating with the type II IFN receptor. Ligand-induced association of both types of IFN receptors results in the phosphorylation of the receptors by Janus kinases subsequently activating STATs (signal transducers and activators of transcription) proteins and additional phosphorylation events that lead to the formation of IFN-inducible transcription factors that bind to IFN response elements presented in IFN-inducible genes. Polypeptides identified as activating the IFN pathways following association with Type 1 and/or Type 2 IFN receptors are considered interferons for purposes of our invention.

Programmed Cell Death Protein 1

Programmed Cell Death Protein 1 ("PD-1"), also known as CD279, is a protein that in humans is encoded by the PDCD1 gene. PD-1 belongs to the immunoglobulin superfamily and functions as a cell surface receptor, binding to two known ligands, PD-L1 and PD-L2.

PD-1 plays an important role in down-regulating the human immune system by preventing the activation of T cells, which in turn reduces autoimmunity and promotes "self-tolerance." The immune regulatory effect of PD-1 is effected by culling active T cells while protecting suppressor T cells. PD-1 promotes apoptosis of antigen-specific T cells in lymph nodes, yet reduces apoptosis in regulatory ("suppressor") T cells.

PD-L1 can be highly expressed in certain tumors. This leads to reduced proliferation of, or even elimination of, immune cells in the tumor, impairing the ability of the patient's innate immune system to recognize cancer cell-surface antigen and combat the cancer cells so identified.

PD-1 is expressed on T cells and pro-B cells. PD-1, functioning as an immune checkpoint, plays an important role in down regulating the immune system by preventing the activation of T-cells, which in turn reduces autoimmunity and promotes self-tolerance. The

inhibitory effect of PD-1 is accomplished through a dual mechanism of promoting apoptosis (programmed cell death) in antigen specific T-cells in lymph nodes while simultaneously reducing apoptosis in regulatory T cells (suppressor T cells).

Programmed death 1 is a type I membrane protein of 268 amino acids. PD-1 is a member of the extended CD28/CTLA-4 family of T cell regulators. The protein's structure includes an extracellular IgV domain followed by a trans-membrane region and an intracellular tail. The intracellular tail contains two phosphorylation sites located in an immune-receptor tyrosine-based inhibitory motif and an immune-receptor tyrosine-based switch motif, which suggests that PD-1 negatively regulates TCR signals. This is consistent with binding of SHP-1 and SHP-2 phosphatases to the cytoplasmic tail of PD-1 upon ligand binding. In addition, PD-1 ligation up-regulates E3-ubiquitin ligases CBL-b and c-CBL that trigger T cell receptor down-modulation. PD-1 is expressed on the surface of activated T cells, B cells, and macrophages, suggesting that compared to CTLA-4, PD-1 more broadly negatively regulates immune responses.

PD-1 has two ligands, PD-L1 and PD-L2, which are members of the B7 family. PD-L1 protein is upregulated on macrophages and dendritic cells (DC) in response to LPS and GM-CSF treatment, and on T cells and B cells upon TCR and B cell receptor signaling, whereas in resting mice, PD-L1 mRNA can be detected in the heart, lung, thymus, spleen, and kidney.

Monoclonal antibodies targeting PD-1 that boost the immune system are being developed for the treatment of cancer. Many tumor cells express PD-L1, an immunosuppressive PD-1 ligand; inhibition of the interaction between PD-1 and PD-L1 can enhance T-cell responses in vitro and mediate preclinical antitumor activity. This is known as immune checkpoint blockade.

One such anti-PD-1 antibody drug, nivolumab, (OPDIVO™, commercially available from Bristol Myers Squibb Co., Princeton, NJ), produced complete or partial responses in non-small-cell lung cancer, melanoma, and renal-cell cancer, in a clinical trial with a total of 296 patients. Colon and pancreatic cancer patients did not have a response. Nivolumab (OPDIVO™, Bristol-Myers Squibb), which also targets PD-1 receptors, was approved in Japan in July 2014 and by the US FDA in December 2014 to treat metastatic melanoma.

Pembrolizumab (KEYTRUDA™ or MK-3475, commercially available from Merck & Co., Rahway, NJ), which also targets PD-1 receptors, was approved by the FDA in Sept 2014 to treat metastatic melanoma. Pembrolizumab has been made accessible to advanced melanoma patients in the UK via UK Early Access to Medicines Scheme (EAMS) in March 2015. It is being used in clinical trials in the US for lung cancer, lymphoma, and mesothelioma. It has had measured success, with little side effects. On October 2, 2015 Pembrolizumab was approved by FDA for advanced (metastatic) non-small cell lung cancer (NSCLC) patients whose disease has progressed after other treatments.

Other drugs in early stage development targeting PD-1 receptors (often referred to as "checkpoint inhibitors"): Pidilizumab (CT-011, Cure Tech), BMS 936559 (Bristol Myers Squibb), MPDL328OA (Roche), and atezolizumab (Amgen).

Combination Therapy

Ihave found that treatment of cancer with interferon - either by administering interferon polypeptide, or by administering an agent which induces cells to express interferon - concomitantly induces expression of PD-1.

Ithus propose improving the efficacy of interferon-based cancer therapy by co-administering interferon with a compound which inhibits the activity of PD-1.

This entails, for example, administering interferon polypeptide intravenously in an amount effective as cancer therapy, and administering a monoclonal antibody checkpoint blockade inhibitor intravenously in an amount effective to prevent an interferon-caused increase in PD-1 expression, and preferably in an amount to reduce the effect of PD-1.

Alternatively, this entails instilling intravesically an agent which induces interferon expression, in an amount effective as cancer therapy, and prophylactically administering a checkpoint blockade inhibitor intravenously in an amount effective to prevent an interferon-caused increase in PD-1 expression, and preferably in an amount to reduce the effect of PD-1. The agent can be an antigenic vaccine (such as a virus, or BCG vaccine or *Mycobacterium* vaccine) which induces interferon expression. Alternatively, the agent can be a transgene vector which transforms a host cell with an expressible interferon transgene. Alternatively, this can be an antigenic virus or bacteria which also delivers an interferon transgene.

EXAMPLE 1 - *IFNα induces PD-L1 and TRAIL expression.*

Interferon-alpha (IFNa) has not been notably effective clinically. Iposited that this might be more effective in the setting of vector-mediated IFNa gene therapy. Several years ago, Ibegan a phase II human clinical trial of INSTILADRIN™ brand adenovirus vector-mediated interferon alpha 2b. In this experiment, Ihad measured the expression of PD-L1, TRAIL, IRF1 and Lamin A in response to exposure to interferon.

Materials & Methods: RT112 and SW780 cells were cultured in media and then exposed to media containing interferon alpha polypeptide. The amount of interferon ranged from zero (control) to 10^4 international units / mL. Gene expression was evaluated by Western blot and quantitative real-time PCR using commercially available antibodies and primers. RNA was isolated from cells in culture with the MIRVANA™ kit (Thermo Fisher). mIRs were profiled in RT112 using TAQMAN™ Array Cards (A and B) (Thermo Fisher).

Whole genome mRNA expression profiling was performed in RT112 and UC3 with Illumina HumanHT_12_v4 BEADCHIP™ arrays (47323 probes).

Results: Results are provided in Figure 1 to 4. In response to exposure to interferon, both cell lines up-regulated PD-L1, TRAIL and IRF1 expression, and had no measurable effect on Lamin A expression. For PD-L1, TRAIL and IRF1 expression, the effect was of different magnitude in the different cell lines. *See* Figure 1, 2 3, 4.

Conclusions: In a panel of cancer cell lines, interferon exposure lead to significant increases in PD-L1 immune checkpoint expression. Ifound this finding surprising because it implied the reason for the failure to-date of the art to use interferon as an effective cancer therapy. While interferon should theoretically be an effective anti-cancer agent, interferon may also up-regulate expression of PD-L1, thus frustrating interferon's therapeutic effect.

EXAMPLE 2 - *IFNα induces PD-L1 expression in a dose-dependent manner.*

Here Ihad measured the expression of immune checkpoint PD-L1, micro-RNA (miR) and mRNA expression profiles after treatment with interferon alpha.

Materials & Methods: RT112, T24, UC3, and UC14 cells were cultured in media and then exposed for 6 hours to either control media, or media containing 1000 IU/ml of interferon alpha polypeptide. Expression of PD-L1 was evaluated by Western blot and quantitative real-time PCR using commercially available antibodies and primers. RNA was isolated from cells in culture with the MIRVANA™ kit (Thermo Fisher). mIRs were profiled in RT112 using TAQMAN™ Array Cards (A and B) (Thermo Fisher). Whole genome mRNA expression profiling was performed in RT112 and UC3 with Illumina HumanHT_12_v4 BEADCHIP™ arrays (47323 probes). All experiments were performed in triplicate to increase statistical reliability.

Results: All cell lines up-regulated the expression PD-L1 in response to exposure to IFNa. This effect was most pronounced in RT112 cells, *see* Figure 5, than in UC3 cells, Figure 6, . In contrast, the expression of three potential *oncomIR* regions was significantly down-regulated after exposure to IFNa in RT112:1233 cells (p = 0.0036), 19b-1# (p = 0.0157), and 222# (p = 0.0061). Analyzing differentially-expressed genes with at least 2-fold differences in log (expression) (false discovery rate <0.001) after IFNa exposure, there were 302 and 181 differentially expressed genes in the RT112 and UC3 cell lines, respectively. Top-ranked IFNa-induced genes in both cell lines included several that had not been previously described in bladder cancer, including IFIT2 (negative regulator of metastasis) and IFI27 (associated with sensitivity to TRAIL). IFNa-induced PD-L1 expression was also demonstrable on the mRNA gene chip with fold-changes paralleling real-time PCR data.

Conclusions: In a panel of cancer cell lines, IFNa exposure lead to significant increases in PD-L1 immune checkpoint expression. Array-based microRNA and mRNA profiling revealed novel potential mediators of IFNa response in bladder cancer. This bladder IFNa profile may be useful as an intermediate endpoint to measure response to adenoviral IFNa gene therapy. Future prediction of PD-L1 expression with IFNa therapy may lead to rational combination treatments utilizing immune checkpoint inhibitors.

EXAMPLE 3 - *Murine interferon induces PD-L1 expression*

Materials and Methods: BBN972 and MB49 #1 (MB49-*luc*) cells were cultured, and then exposed to media containing from 0 (zero) to 1 x 10^4 international units of murine interferon. Subsequent expression of PD-L1 and (as a control) actin were measured.

Results: Murine interferon had no effect on the expression of actin in either cell line. *See* Figures 11, 12. In contrast, Murine interferon had a marked, dose-dependent effect on PD-L1 expression. *See* Figures 9, 10.

Conclusions: These data show that the effect of interferon on PD-L1 expression is not limited to human interferon alpha 2a, nor indeed to human interferon. Rather, the effect of interferon on expression of PD-L1 appears to be generic to interferon generally.

EXAMPLE 4 - *Polyinosinic:polycytidylic acid (Poly I:C) induces PD-L1*

Materials & Methods: The foregoing data indicate that interferon induces PD-L1 expression, does so in a dose-dependent manner, does so quickly, and does so apparently in response to interferon from different species. Given the effect regardless of the animal species from which the interferon was taken, Ihypothesized that the effect might not be limited to interferon, and might be more generally provoked by immune stimulants of other types. To test the concept, Ihad evaluated Polyinosinic:polycytidylic acid (often abbreviated "poly I:C"). Poly I:C is an immunostimulant. It is used in the form of its sodium salt to simulate viral infections. Poly I:C is structurally similar to double-stranded RNA. dsRNA is present in some viruses. Ihad Poly I:C administered via intra-peritoneal injection to laboratory mice with implanted *plc* or *ulc* tumors.

Results. Figure 13 shows that control mice (n = 3) showed a de minimus baseline measure of serum interferon a. In contrast, intra-peritoneal injection of Poly I:C produces a time-dependent increase in serum interferon a. Figure 14 shows results of intra-tumoral injection of Poly I:C at 6 hours. The data (n = 1 for each series) show that intra-tumor interferon a increases significantly in *plc* tumors, increases somewhat in *ulc* tumors, and does not measurably increase in control tumors. Figure 15 shows that Poly I:C (500 mcg) also induces (at 24 hours) PD-L1 expression intra-tumorally (Mann Whitney p = 0.0495).

Conclusions: These data indicate that PD-L1 expression is induced not merely by interferon, but by Poly I:C, a compound which mimics dsRNA and which induces interferon expression.

393

394 EXAMPLE 5 - *Interferon Viral Gene Therapy Induces PD-L1 In Humans*

395 Materials & Methods: These data are taken from a human Phase II human clinical
396 trial for INSTILADRIN™ replication-deficient adenoviral gene therapy vector carrying a
397 human interferon alpha 2b transgene in patients unresponsive to or refractory after BCG
398 therapy. That study plan has been published and is incorporated here by reference.

399 Results: Figure 16 shows RNA expression in eight (8) treatment cycles in humans
400 treated with INSTILADRIN™ recombinant replication-deficient adenovirus gene therapy
401 vector carrying a human interferon alpha 2B transgene. Odd (white color coded) columns
402 measure RNA transcription before treatment; even (light blue color coded) columns measure
403 after. RNA amounts are shown quantitatively, light green showing the least and light red the
404 most. Columns 1 and 2 show PD-L1 RNA increasing from -2 before treatment to +2 after.
405 Columns 3 and 4 similarly show PD-L1 RNA increasing from -2 before treatment to +3 after.
406 In all, one third of the treatment pair show a significant increase in PD-L1 expression after
407 treatment. Treatment also up-regulated other immune checkpoint markers.

408 Conclusions: These data show that one third of patients demonstrate induction of T-
409 cell and immune checkpoint markers (including PD-L1) after treatment with interferon gene
410 therapy.

411

412 EXAMPLE 6 - *Combination Therapy Increases Survival*

413 Materials & Methods: Female laboratory rats were inoculated with tumor cells, and
414 the cells allowed to deveop into measurable tumors. The rats were then treated with saline
415 (control), IgG (as a control), anti-PD1 monoclonal antibody (monotherapy), Poly I:C
416 (monotherapy to induce interferon expression) and a combination of Poly I:C and anti-PD1
417 monoclonal antibody (combination therapy).

Results: Figure 17 shows MB49 tumor size vs time, for subcutaneous C57BL6/J tumors (n = 5 female mice per group). Treatment is 200 mcg q3 days starting on day 10 after tumor implant. Error bard represent SEM. The highest (yellow) line, showing the largest tumor volume at day 40, is control group (all groups n = 5, female-only). The next lowest (blue) line is the IgG control. The next lowest (red) line is Poly I:C. The next lowest (green) line is anti-PD1 Monoclonal antibody. The lowest (black) line, laying on the X axis itself, is combination therapy.

Figure 18 shows a Kaplan-Meyer survival curve for female mice with inoculated tumors, treated with saline (lowermost line), IgG (next higher line), anti-PD1 monoclonal antibody (next higher line), Poly I:C (next higher line) and a combination of Poly I:C and anti-PD1 monoclonal antibody (highest line). These data show that combining an interferon-inducing agent (Poly I:C) and a PD1 inhibitor (an anti-PD1 monoclonal antibody) increases survival significantly: at 50 days, ~20% of control animal remain alive, 50% of Poly I:C animals remain alive, and 100% of combination treated animals remain alive.

Figure 19 compares normalized (mean +/- SD) radiance over time in male mice. Using a log-rank test, these data show combination therapy superior to IgG control ($p = 0.06$), superior to Poly I:C monotherapy ($p = 0.32$), and superior to anti-PD1 monoclonal antibody ($p = 0.14$).

Figure 20 shows "survival portions," i.e., data showing the survival of propensity to survive, over time.

Conclusions: These data show combination therapy synergistically effective, imparting a more than merely additive effect.

EXAMPLE 7 - *Superficial Spreading Melanoma*

Materials & Methods: A human patient diagnosed with superficial spreading melanoma is treated by wide local excision and sentinel node biopsy to confirm lack of spread of the disease to the lymph system or distal organs. The patient is then treated with a combination of INSTILADRIN™ and KEYTRUDA™. Treatment is initiated as soon as practical after surgical resection.

INSTILADRIN™ brand adenovirus is a replication-deficient, recombinant adenoviral gene therapy vector bearing an interferon alpha 2b transgene. The manufacture of such gene therapy vectors is described in, *e.g.*, Muralidhara Ramachandra *et al., Selectively Replicating Viral Vector*, United States Letters Patent No. 7691370. The isolation of interferon transgenes is described in *e.g.*, Charles Weissmann, *DNA Sequences, Recombinant DNA Molecules and Processes for Producing Human Interferon-Like Polypeptides* , United States Letters Patent No. 6835557.

KEYTRUDA™ brand pembrolizumab is a humanized monoclonal anti-programmed cell death-1 (PD-1) antibody (IgG4/kappa isotype with a stabilising sequence alteration in the Fc region).

INSTILADRIN™ is provided in single-dose vials. One dose of INSTILADRIN™ is reconstituted in sterile saline for injection and administered subcutaneously locally to the excision site. Administration is repeated once every four weeks. One vial of KEYTRUDA™ powder contains 50 mg of pembrolizumab. KEYTRUDA™ is administered as an intravenous infusion over 30 minutes, repeated every 3 weeks, and patients are treated until disease progression or unacceptable toxicity. Atypical responses (i.e., an initial transient increase in tumour size or small new lesions within the first few months followed by tumour shrinkage) may be observed. It is preferred to continue treatment for clinically-stable patients with initial evidence of disease progression until disease progression is confirmed.

Test subjects are enrolled and then assigned to a treatment group: excision followed by KEYTRUDA™ only, excision followed by INSTILADRIN™ only, excision followed by KEYTRUDA™ and INSTILADRIN™ concomitantly, excision followed by INSTILADRIN™ and NSAID (a COX-2 inhibitor), and excision followed by KEYTRUDA™ and INSTILADRIN™ and NSAID concomitantly.

Results: The primary efficacy outcome measures are progression free survival (as assessed by *e.g.*, an Integrated Radiology and Oncology Assessment review using Response Evaluation Criteria in Solid Tumours [RECIST]), overall survival, and sentinel node biopsy. Other efficacy outcome measures may be overall response rate and response duration. Subsequent sentinel node biopsy is expected to show no spread of the disease.

Iexpect that administration of INSTILADRIN™ with COX-2 inhibitor will demonstrate superior efficacy to INSTILADRIN™ only. Iexpect that administration of KEYTRUDA™ and INSTILADRIN™ concomitantly will demonstrate superior efficacy outcome measures as compared to administration of either agent alone, and Iexpect this benefit to be more than merely additive. Iexpect that administration of KEYTRUDA™ and INSTILADRIN™ and NSAID concomitantly will demonstrate superior efficacy outcome measures as compared to administration of of KEYTRUDA™ alone or INSTILADRIN™ and NSAID alone, and Iexpect this benefit to be more than merely additive.

EXAMPLE 8 - *Superficial Spreading Melanoma*

Materials & Methods: KEYTRUDA™ as in the foregoing example.

As a source of interferon, SYLATRON™ PEG-ylated interferon alpha 2b, administered subcutaneously at 6 mcg/kg once weekly for 8 doses (induction), followed by 3 mcg/kg once weekly for up to 5 years (maintenance). If SYLATRON™ dosage modification is required during weeks 1–8 of treatment (induction) because of adverse reactions, a 3-step

decrease from original dosage (6 mcg/kg once weekly) is preferred (i.e., decrease dosage to 3 mcg/kg once weekly; if needed, decrease to 2 mcg/kg once weekly; then, if needed, further decrease to 1 mcg/kg once weekly). If dosage modification required during weeks 9–260 of treatment (maintenance) because of adverse reactions, a 2-step decrease from original dosage (3 mcg/kg once weekly) recommended (i.e., decrease dosage to 2 mcg/kg once weekly; if needed, decrease to 1 mcg/kg once weekly).

Test subjects are enrolled and then assigned to a treatment group: excision followed by KEYTRUDA™ only, excision followed by SYLATRON™ only, excision followed by KEYTRUDA™ and SYLATRON™ concomitantly, excision followed by SYLATRON™ and NSAID (a COX-2 inhibitor), and excision followed by KEYTRUDA™ and SYLATRON™ and NSAID concomitantly.

Results: The primary efficacy outcome measures are progression free survival (as assessed by *e.g.*, an Integrated Radiology and Oncology Assessment review using Response Evaluation Criteria in Solid Tumours [RECIST]), overall survival, and sentinel node biopsy. Other efficacy outcome measures may be overall response rate and response duration. Subsequent sentinel node biopsy is expected to show no spread of the disease.

Iexpect that administration of SYLATRON™ with COX-2 inhibitor will demonstrate superior efficacy to SYLATRON™ only. Iexpect that administration of KEYTRUDA™ and SYLATRON™ concomitantly will demonstrate superior efficacy outcome measures as compared to administration of either agent alone, and Iexpect this benefit to be more than merely additive. Iexpect that administration of KEYTRUDA™ and SYLATRON™ and NSAID concomitantly will demonstrate superior efficacy outcome measures as compared to administration of of KEYTRUDA™ alone or SYLATRON™ and NSAID alone, and Iexpect this benefit to be more than merely additive.

EXAMPLE 9 - *Non-Small Cell Lung Cancer*

Materials & Methods: Pharmaceutical Agents as per Example 7 above. Human test subjects are diagnosed as having Non-Small-Cell Lung Carcinoma. Patients are screened according to Greene, Frederick L., *Cancer Staging Manual* (American Joint Committee on Cancer, publ., 6th edition) to assure that comparable test subjects have comparable disease. Test subjects are screened for treatment based on the tumor expression of PD-L1, expression confirmed by a validated test.

The recommended dose of KEYTRUDA is: 200 mg for NSCLC that has not been previously treated with chemotherapy, and 2 mg/kg for NSCLC that has been previously treated with chemotherapy or for melanoma.

INSTILADRIN™ is administered by intra-pleaural infusion. This method is illustrated in United States Patent publication US2014/17202 at Figure 2.

Test subjects are enrolled and then assigned to a treatment group: KEYTRUDA™ only, INSTILADRIN™ only, KEYTRUDA™ and INSTILADRIN™ concomitantly, INSTILADRIN™ and NSAID (a COX-2 inhibitor), and KEYTRUDA™ and INSTILADRIN™ and NSAID concomitantly.

Results: The primary efficacy outcome measures are progression free survival, overall survival, and sentinel node biopsy. Other efficacy outcome measures may be overall response rate and response duration. Subsequent sentinel node biopsy is expected to show no spread of the disease.

Iexpect that administration of INSTILADRIN™ with COX-2 inhibitor will demonstrate superior efficacy to INSTILADRIN™ only. Iexpect that administration of KEYTRUDA™ and INSTILADRIN™ concomitantly will demonstrate superior efficacy outcome measures as compared to administration of either agent alone, and Iexpect this benefit to be more than merely additive. Iexpect that administration of KEYTRUDA™ and

INSTILADRIN™ and NSAID concomitantly will demonstrate superior efficacy outcome measures as compared to administration of of KEYTRUDA™ alone or INSTILADRIN™ and NSAID alone, and Iexpect this benefit to be more than merely additive.

Summary

The above Examples discuss treating certain cancers. Our discovery, however, may be more generally used to treat any condition which benefits from interferon signaling, and which suffers from over-expression of CD279.

In the appended claims, Iuse the term "treat" not to require complete cure, but to ameliorate. For example, "treating" cancer may be achieved by completely eliminating the cancer, and also by, for example, slowing tumor growth, reducing the risk of mortality or slowing disease progression when compared to patients who do not have such treatment.

Given our disclosure here, the artisan can readily see specific applications or variants of it. For example, while the above discussion mentions specific species of human interferon, other species and interferon derivatives or analogs which function similarly will provide the same benefit. Thus, Iintend the legal coverage of our patent to be determined not by the Examples Idiscuss, but by the appended legal claims and permissible equivalents thereof.

When the appended legal claims refer to treating at about "the same time," *see e.g.*, original claim 3, this requires the two compounds work in the patient at the same time. It does not require contemporaneous administration. Thus, one could administer the first agent a week after administering the second agent, if the effect of the second agent persistes for at least a week.

CLAIMS

We claim:

1. A method of treating a human patient with interferon comprising:

a. diagnosing in a human patient a condition which may be treated with interferon, and then

b. administering a first agent, the first agent able to increase the human patient's level of interferon in an amount sufficient to affect the function of a human immune system checkpoint, whereby the checkpoint would decrease an immune function, and

c. administering to the human a second agent which affects the function of the same human immune system checkpoint, the second agent administered in an amount effective to substantially ameliorate the decrease in immune function which the first agent would have on said checkpoint,

whereby the second compound substantially ameliorates the decrease in the immune function which would be caused by the first agent.

2. A method consisting essentially of the method of claim 1.

3. The method of claim 1, where the patient is treated with the first agent and the second agent at about the same time.

4. The method of claim 1, where the first agent comprises exogenously-produced interferon polypeptide.

5. The method of claim 1, where the first agent comprises an agent which induces the patient to endogenously express interferon.

6. The method of claim 5, where the first agent is a vector carrying an expressible interferon transgene.

7. The method of claim 5, where the first agent is selected from the group consisting of: microbial antigen, viral antigen and microbial or viral antigen analog.

8. The method of claim 7, where the first agent comprises viral antigen analog comprising Poly I:C.

9. The method of claim 7, where the first agent comprises bacterial antigen.

10. The method of claim 7, where the first agent comprises viral antigen.

11. The method of claim 10, where the first agent comprises antigenic virus.

12. The method of claim 1, where the human patient's level of interferon is increased in an amount sufficient to increase the function of an inhibitory human immune system checkpoint.

13. The method of claim 12, where the inhibitory checkpoint is selected from the group consisting of: Programmed Cell Death Protein 1 and Cytotoxic T-Lymphocyte-Associated Protein 4.

14. The method of claim 13, where the inhibitory checkpoint comprises Programmed Cell Death Protein 1 and the second agent binds to Programmed Cell Death Protein 1 or Programmed Cell Death Protein 1 ligand.

15. The method of claim 1, where the condition comprises cancer.

16. A agent which affects the function of a human immune system checkpoint whereby the checkpoint does not suppress a human immune function, said agent for use in eliminating an immune-suppressing effect of interferon on a human patient, said agent provided in an amount effective to reduce the immune-suppressing effect of interferon.

17. A method for improving the efficacy of interferon as a human therapeutic, the method comprising:

a. diagnosing in a human patient a condition which may be treated with interferon, and then

b. administering to the human a second agent which inhibits an inhibitory human immune system checkpoint, the agent administered in an amount sufficient to reduce the activity of the checkpoint on decreasing immune activity, and

c. administering to the human a first agent which increases the human patient's level of interferon in an amount effective to treat the condition.

18. A method consisting essentially of:

a. administering to a human a second agent which inhibits an inhibitory human immune system checkpoint, and

b. administering to the human a first agent which increases the human patient's level of interferon.

19. The method of claim 18, the second agent administered in an amount sufficient to reduce the activity of the checkpoint.

AMENDED CLAIMS
received by the International Bureau on 14 June 2017 (14.06.2017)

1. A method of treating a human patient with interferon comprising:

a. diagnosing in a human patient a condition which may be treated with interferon, and then

b. administering a first agent, the first agent able to increase the human patient's level of interferon in an amount sufficient to affect the function of a human immune system checkpoint, whereby the checkpoint would decrease an immune function, and

c. administering to the human a second agent which affects the function of the same human immune system checkpoint, the second agent administered in an amount effective to substantially ameliorate the decrease in immune function which the first agent would have on said checkpoint,

whereby the second compound substantially ameliorates the decrease in the immune function which would be caused by the first agent.

2. A method consisting essentially of the method of claim 1.

3. The method of claim 1, where the patient is treated with the first agent and the second agent at about the same time.

4. The method of claim 1, where the first agent comprises exogenously-produced interferon polypeptide.

5. The method of claim 1, where the first agent comprises an agent which induces the patient to endogenously express interferon.

6. The method of claim 5, where the first agent is a vector carrying an expressible interferon transgene.

7. The method of claim 5, where the first agent is selected from the group consisting of: microbial antigen, viral antigen and microbial or viral antigen analog.

8. The method of claim 7, where the first agent comprises viral antigen analog comprising Poly I:C.

9. The method of claim 7, where the first agent comprises bacterial antigen.

10. The method of claim 7, where the first agent comprises viral antigen.

11. The method of claim 10, where the first agent comprises antigenic virus.

12. The method of claim 1, where the human patient's level of interferon is increased in an amount sufficient to increase the function of an inhibitory human immune system checkpoint.

13. The method of claim 12, where the inhibitory checkpoint is selected from the group consisting of: Programmed Cell Death Protein 1 and Cytotoxic T-Lymphocyte-Associated Protein 4.

14. The method of claim 13, where the inhibitory checkpoint comprises Programmed Cell Death Protein 1 and the second agent binds to Programmed Cell Death Protein 1 or Programmed Cell Death Protein 1 ligand.

15. The method of claim 1, where the condition comprises cancer.

16. A agent which affects the function of a human immune system checkpoint whereby the checkpoint does not suppress a human immune function, said agent for use in eliminating an immune-suppressing effect of interferon on a human patient, said agent provided in an amount effective to reduce the immune-suppressing effect of interferon.

17. A method for improving the efficacy of interferon as a human therapeutic, the method comprising:

a. diagnosing in a human patient a condition which may be treated with interferon, and then

b. administering to the human a second agent which inhibits an inhibitory human immune system checkpoint, the agent administered in an amount sufficient to reduce the activity of the checkpoint on decreasing immune activity, and

c. administering to the human a first agent which increases the human patient's level of interferon in an amount effective to treat the condition.

18. A method consisting essentially of:

a. administering to a human a second agent which inhibits an inhibitory human immune system checkpoint, and

b. administering to the human a first agent which increases the human patient's level of interferon.

19. The method of claim 18, the second agent administered in an amount sufficient to reduce the activity of the checkpoint.

20. The method of claim 19, wherein the second agent induces the patient to endogenously express interferon, and wherein the second agent comprises a vector carrying an expressible interferon transgene.

STATEMENT UNDER ARTICLE 19 (1)

This Statement corrects certain errors in the 26 April *Written Opinion*. Applicant cannot address every error in the 26 April *Written Opinion* because PCT Rule 46 limits the word count of this response. That said, Applicant respectfully notes:

Broad Institute and the instant invention address the same problem. The two disclosures, however, pose different solutions. Broad Institute proposes genotyping the patient and then making a personalized vaccine. *See e.g.*, Figure 1. In contrast, the instant disclosure proposes administering an agent which increases interferon. *See e.g.*, claim 1(b). That agent need not be personalized.

Broad Institute's personalized approach might enable reduced dosages, thus reducing adverse side effects. In contrast, the instant approach is far less expensive and, by dispensing with genotyping and personalized vaccines, is useable outside major international research hospitals.

The two disclosures teach different solutions. It is therefore not surprising that Broad Institute fails to teach every element of the instant claims.

For example, immune checkpoints are classified as either stimulatory or inhibitory. Broad Institute "fails to explicitly state wherein the human patient's level of interferon is increased in an amount sufficient to increase the function of an inhibitory human immune system checkpoint." *See Written Opinion* at Box V sheet 3, Claim 12. The Examiner correctly notes this. *Id.* Broad Institute, however, similarly fails to teach increasing interferon enough to increase the function of a stimulatory checkpoint. The Examiner does not appear to dispute this. *See id.* Broad Institute therefore fails to teach every limitation of Claim 1 or Claim 17. Broad Institute therefore cannot destroy novelty.

In response, the Examiner notes that "high doses of TNF, Para. [00353]" may suppress the immune system. *See Written Opinion* at Box V sheet 1, Claim 11. This may be correct, but is not relevant: TNF does not increase interferon. *See* [00353].

Alternatively, the Examiner says, "interferon-a [IFNα] [] provides a measurable but marginal benefit, Para. [2009]." Broad Institute, however, does not have any "Para. [2009]."

Alternatively, the Examiner says, "IFN-γ secretion occurs as a result … mitogenic stimuli by CD4+ and/or CD8 T-cells, Para. [00492]." This is correct, but irrelevant. [00492] explains how IFN-γ secretion occurs. [00492], however, fails to teach raising IFN-γ enough to change immune checkpoint function.

Broad Institute therefore fails to teach every limitation of Claim 1 or Claim 17.

Regarding claim 12, the Examiner correctly notes that BALD (2014) Figure 6C teaches "melanoma-bearing mice treated with poly[I:C] … showed increased numbers of PD-1 expressing CD8+ T cells." This is correct, but incomplete. Figure 6C shows that after treatment with poly[I:C], 10.4% of CD8+ T cells express PD-1. This means that after treatment, 89.6% of cells ***do not express*** PD-1. 89.6% of T cells would seem adequate to maintain complete human immune function. Figure 6C thus fails to show "decreasing an immune function" as required by the claims.

Broad Institute combined with BALD (2014) thus fails to teach every limitation of the claims. The combination therefore cannot destroy inventive step.

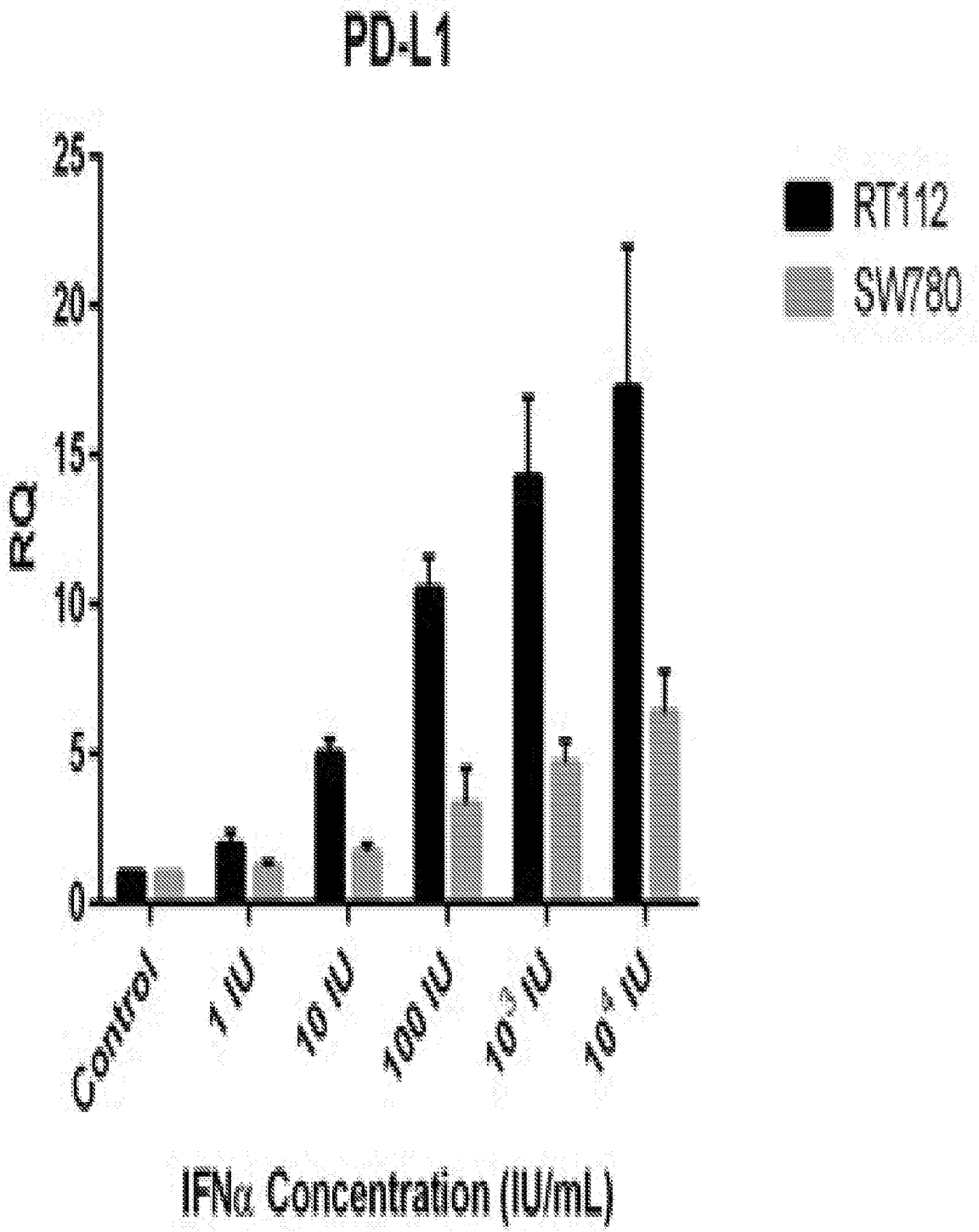
PD-L1
RT112
SW780
RQ
25
20
15
10
5
0
Control
1 IU
10 IU
100 IU
10^3 IU
10^4 IU
IFNα Concentration (IU/mL)

Figure 1

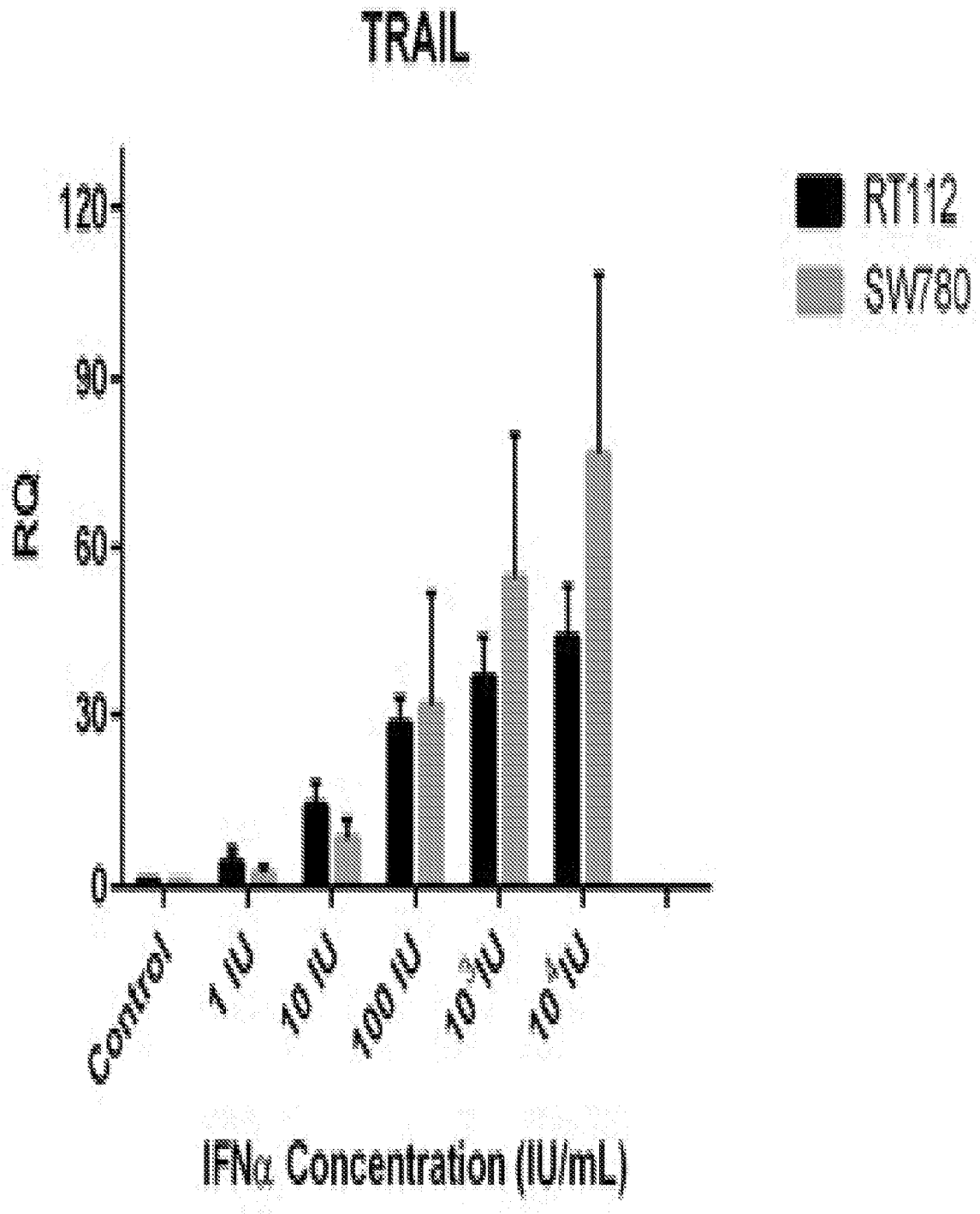
TRAIL
RT112
SW780
RQ
120
90
60
30
0
Control
1 IU
10 IU
100 IU
10^3 IU
10^4 IU
IFNα Concentration (IU/mL)

Figure 2

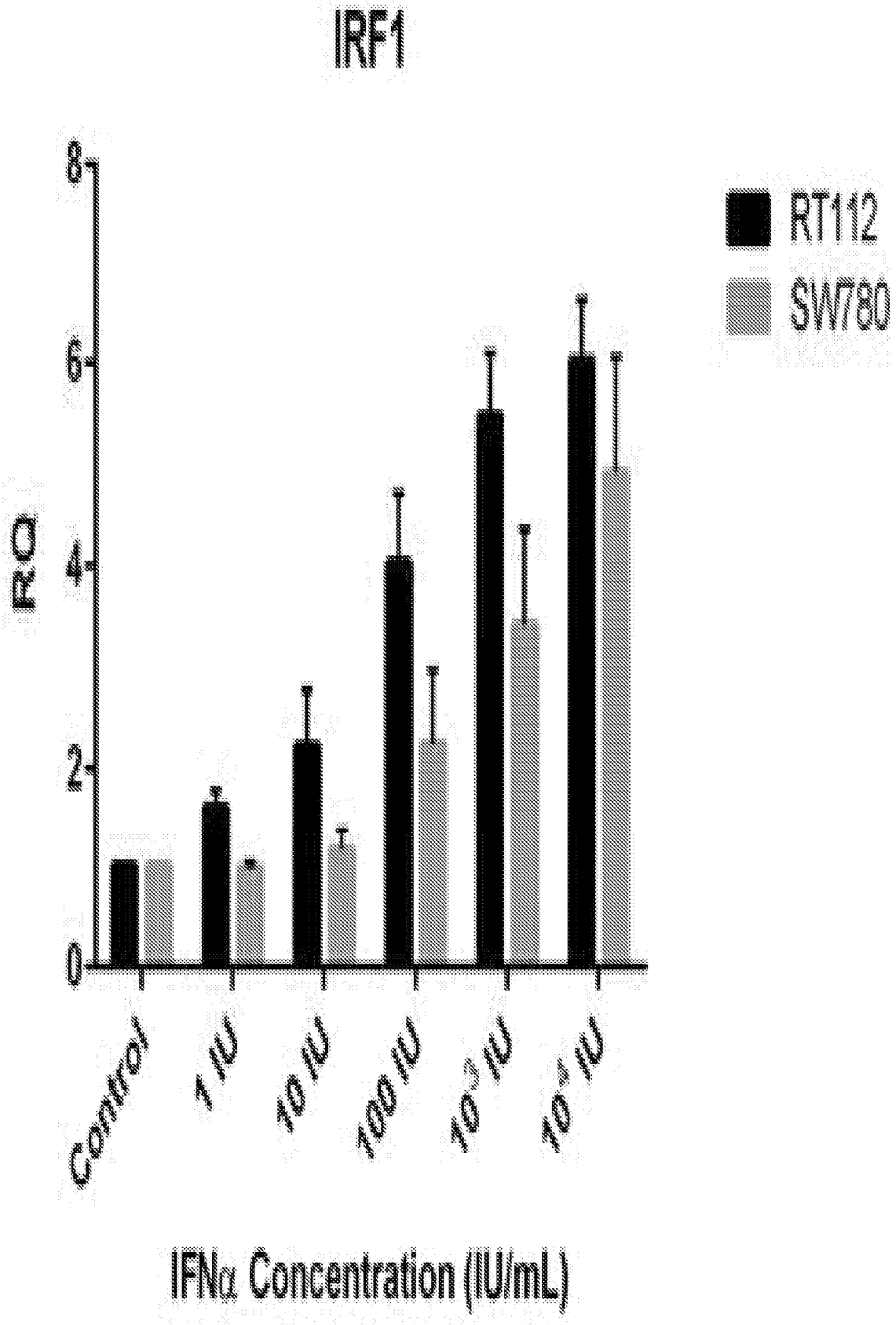
IRF1
RT112
SW780
RQ
8
6
4
2
0
Control
1 IU
10 IU
100 IU
10^3 IU
10^4 IU
IFNα Concentration (IU/mL)

Figure 3

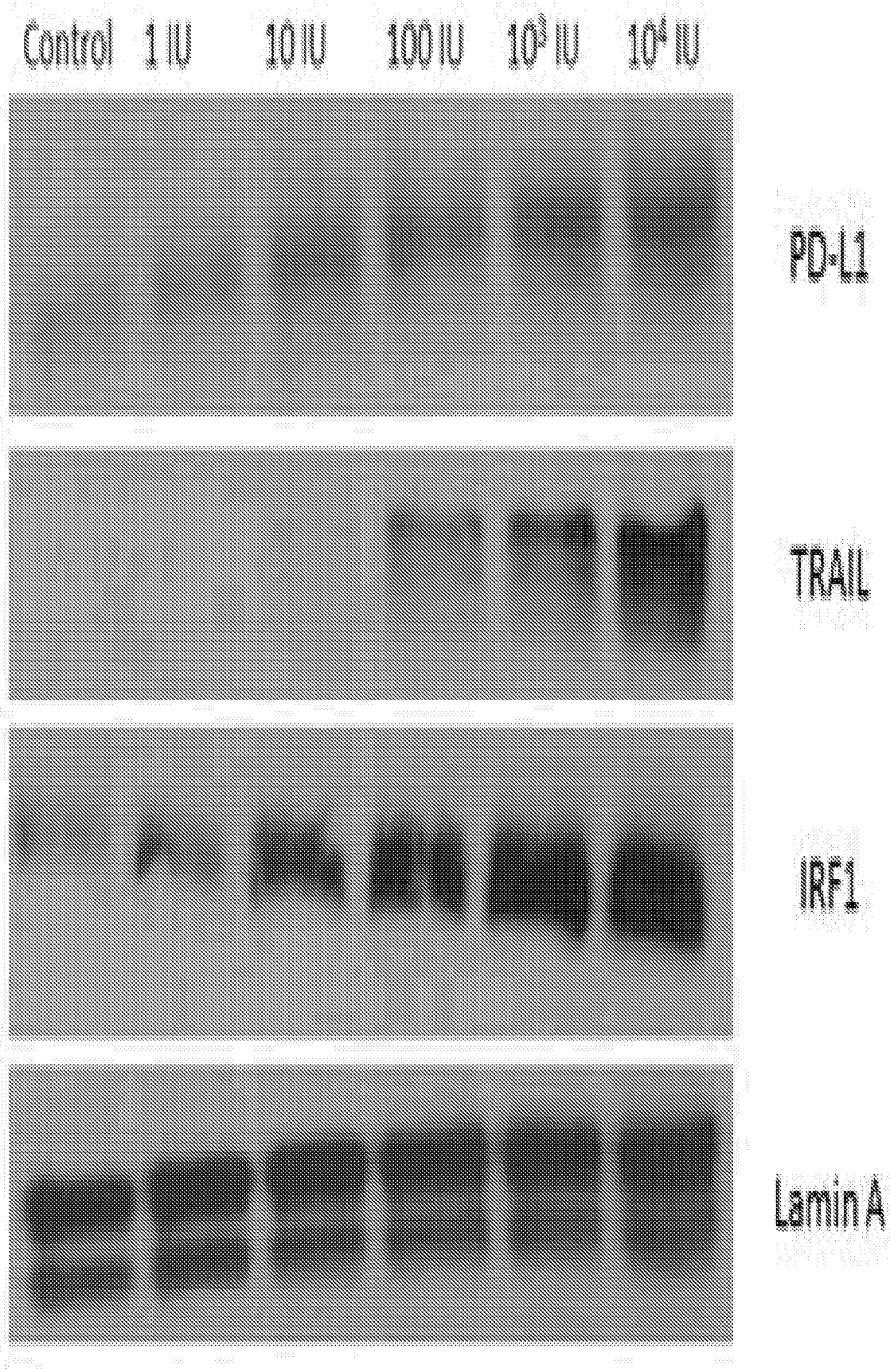

Figure 4

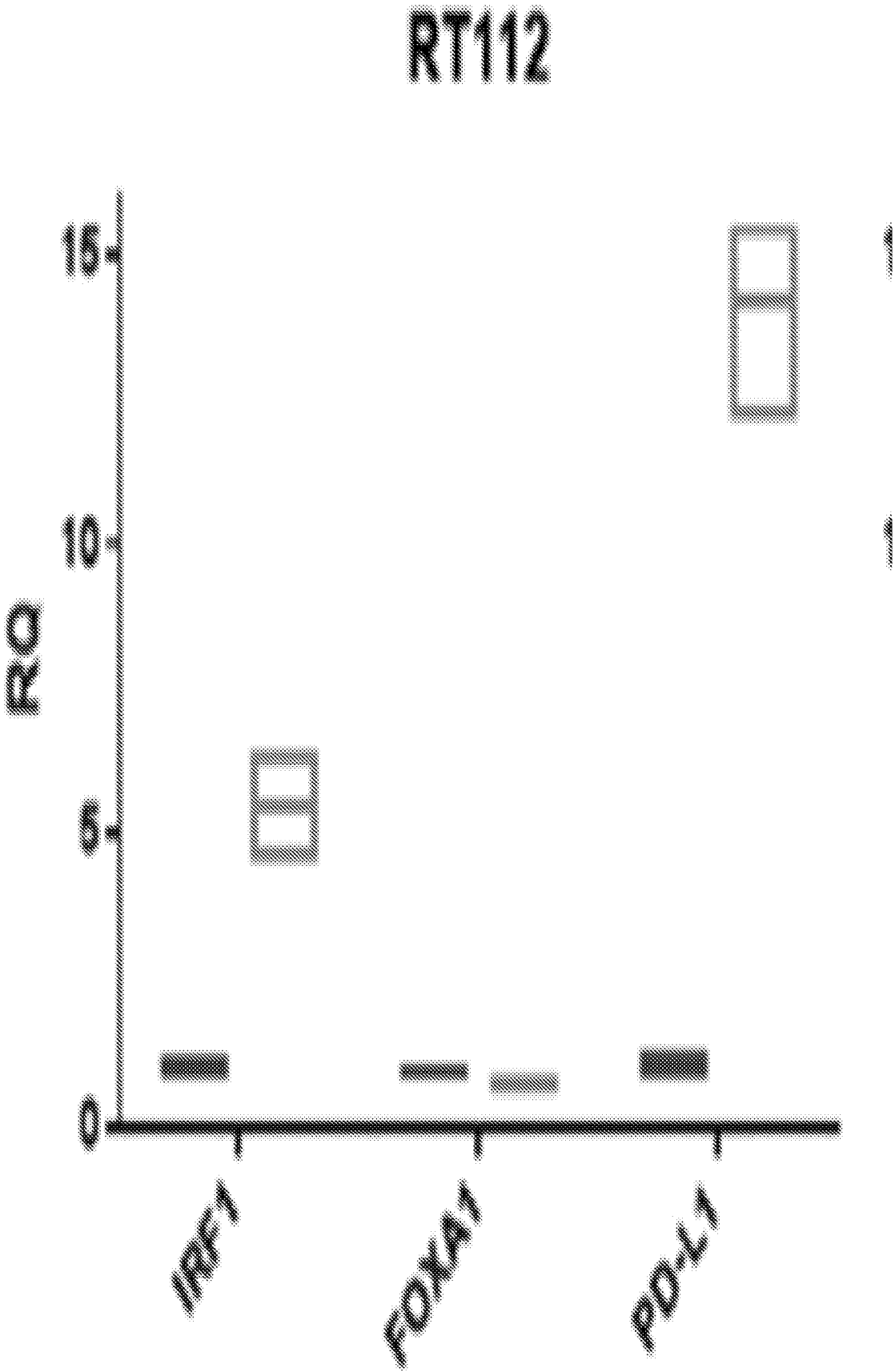

Figure 5

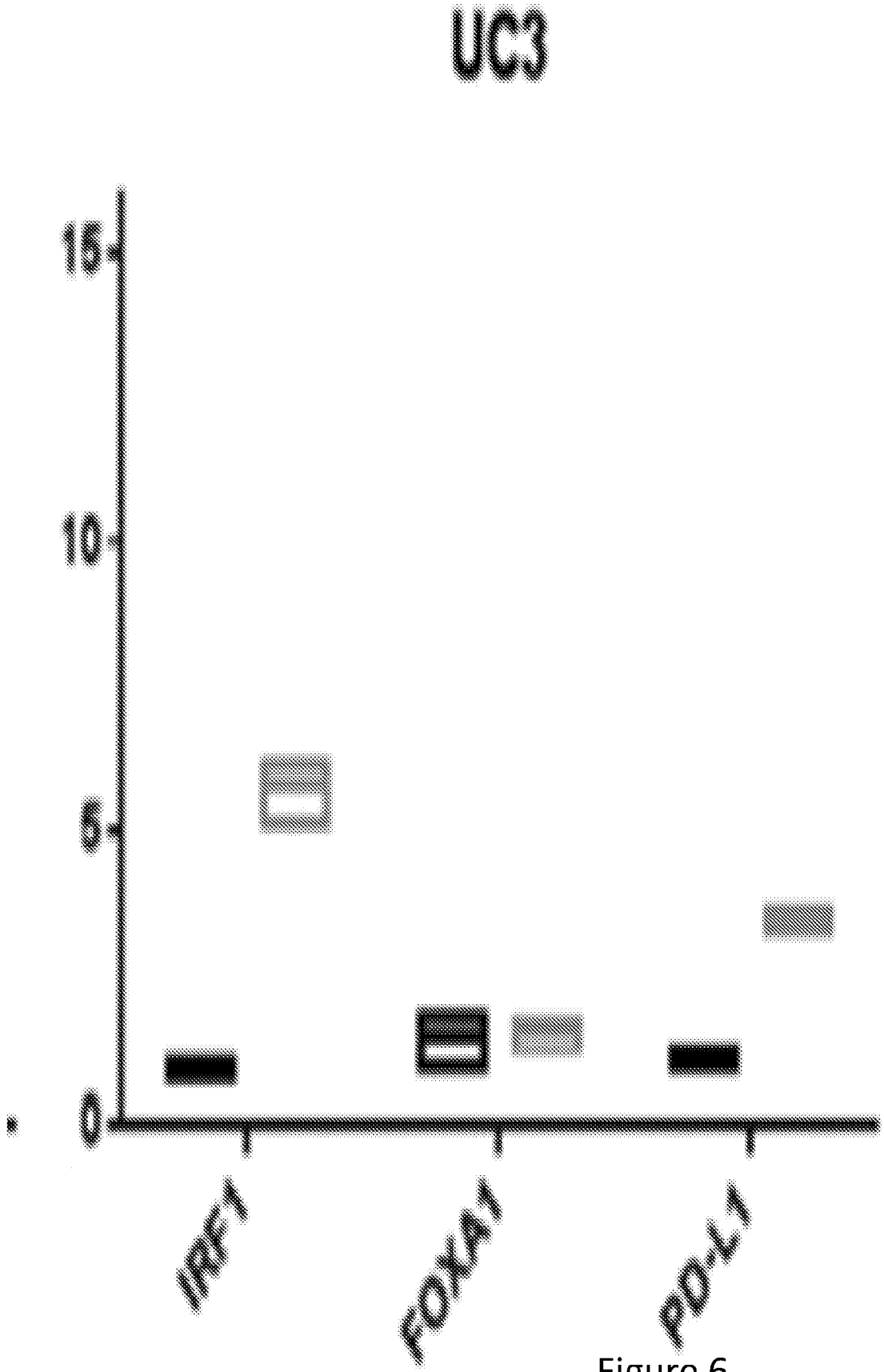

Figure 6

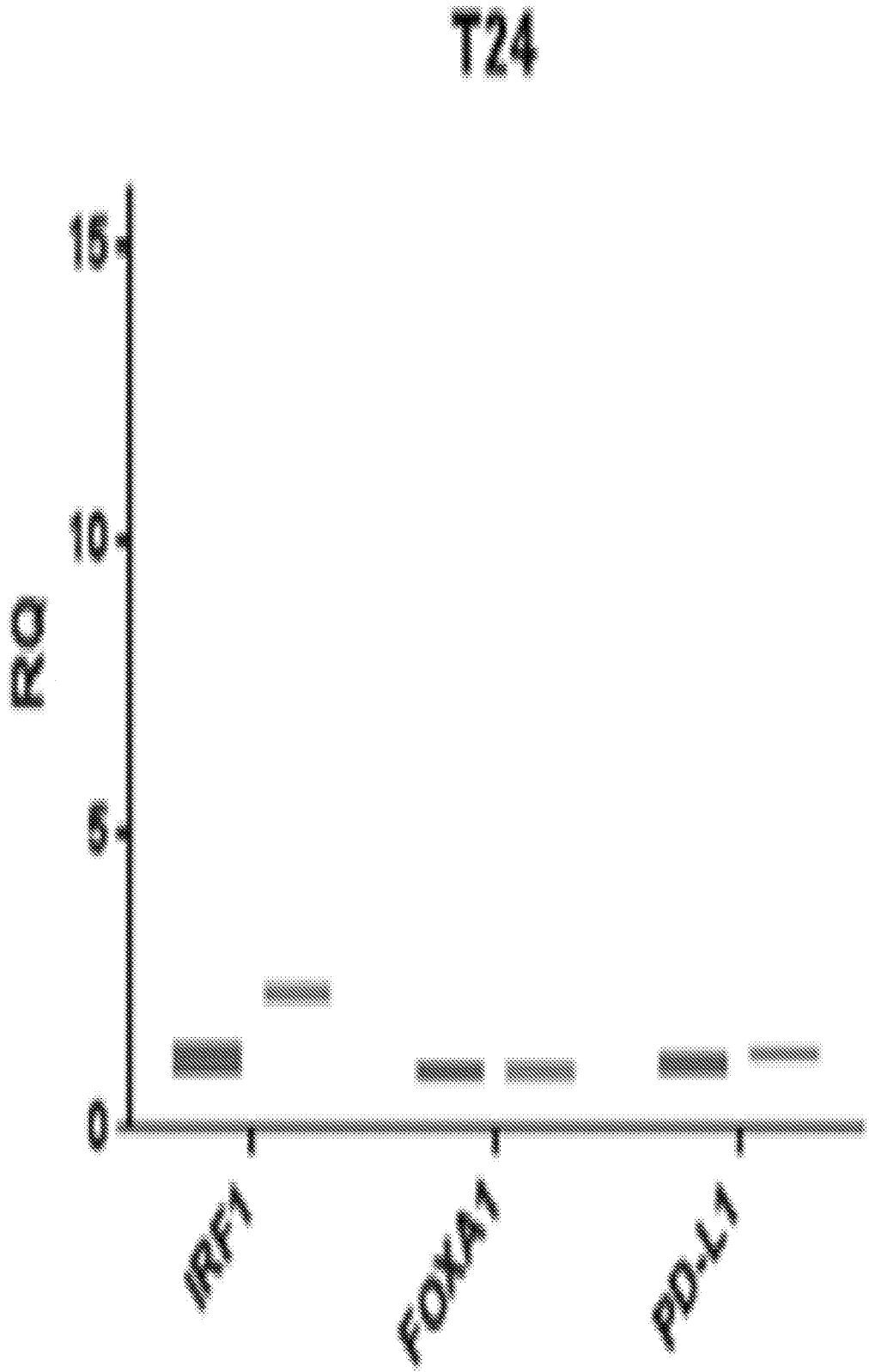

Figure 7

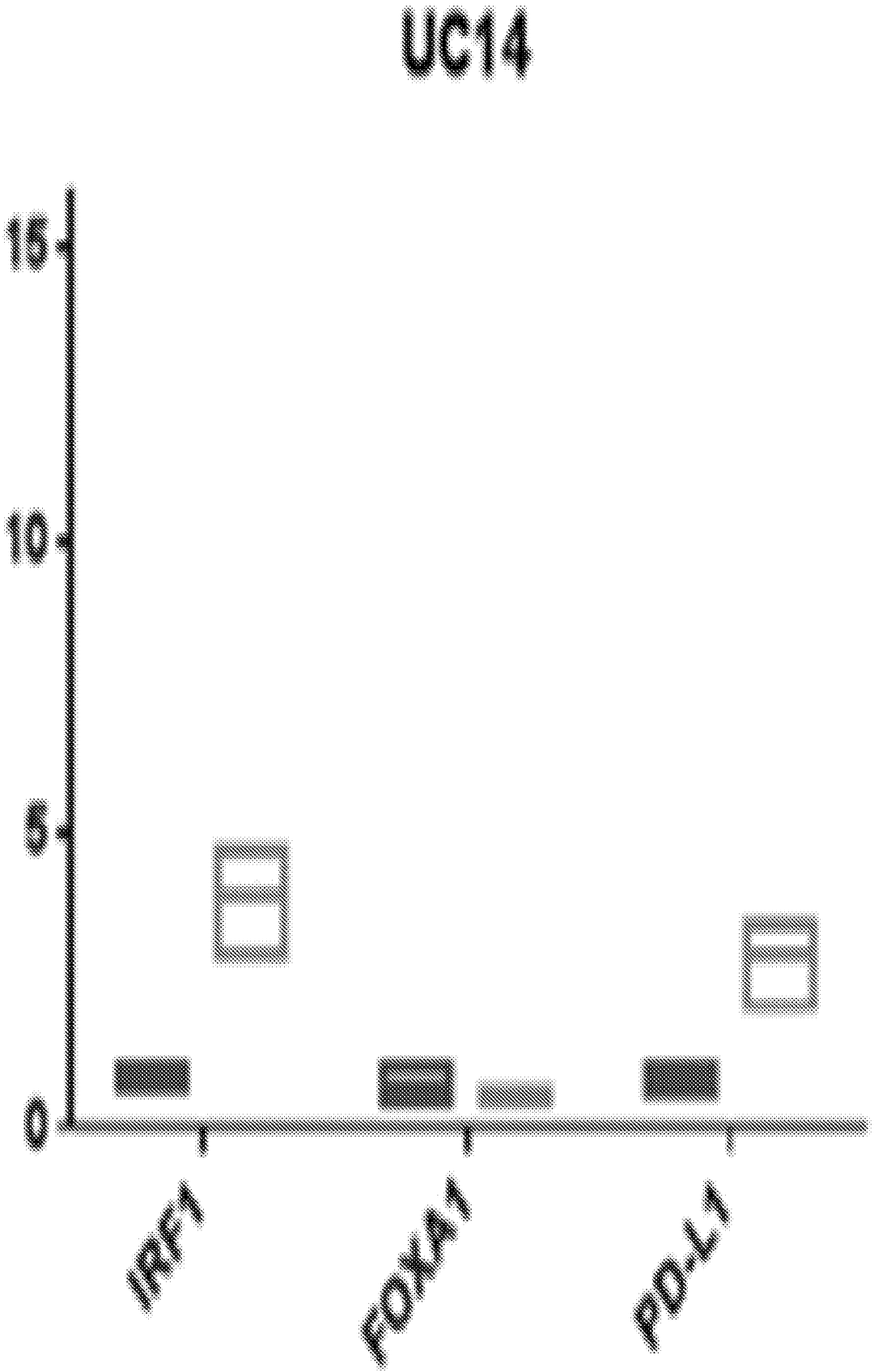

Figure 8

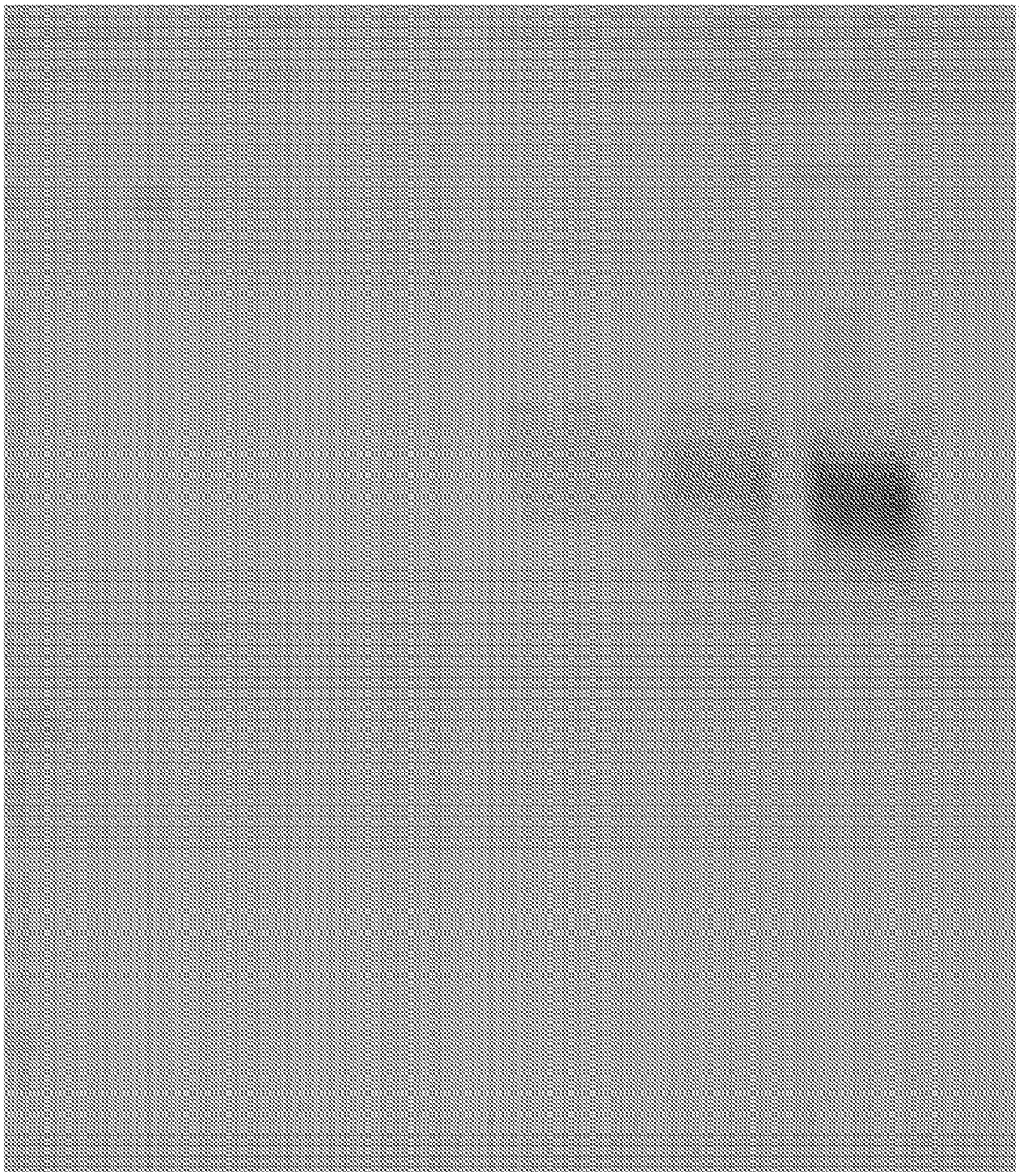

Figure 9

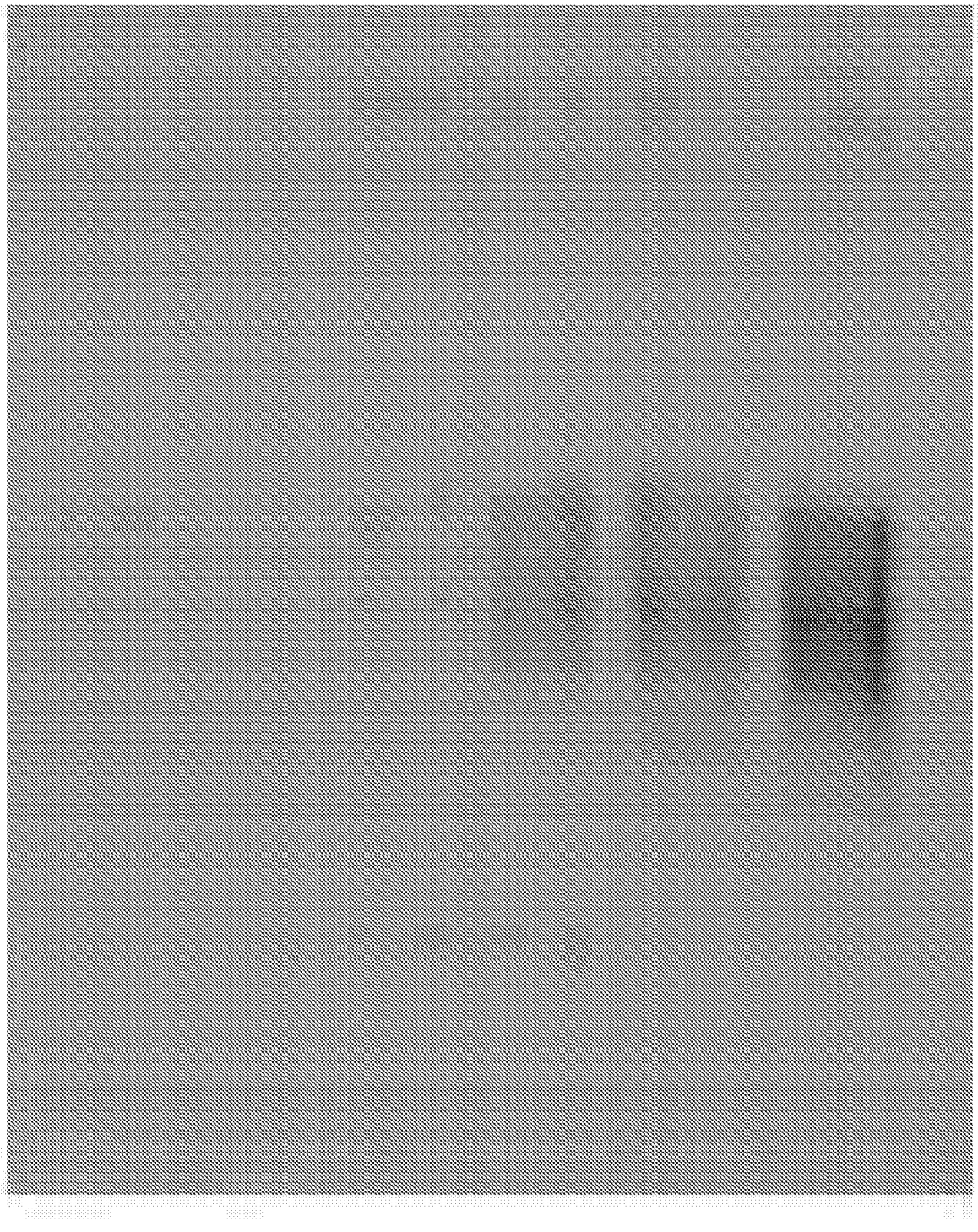

Figure 10

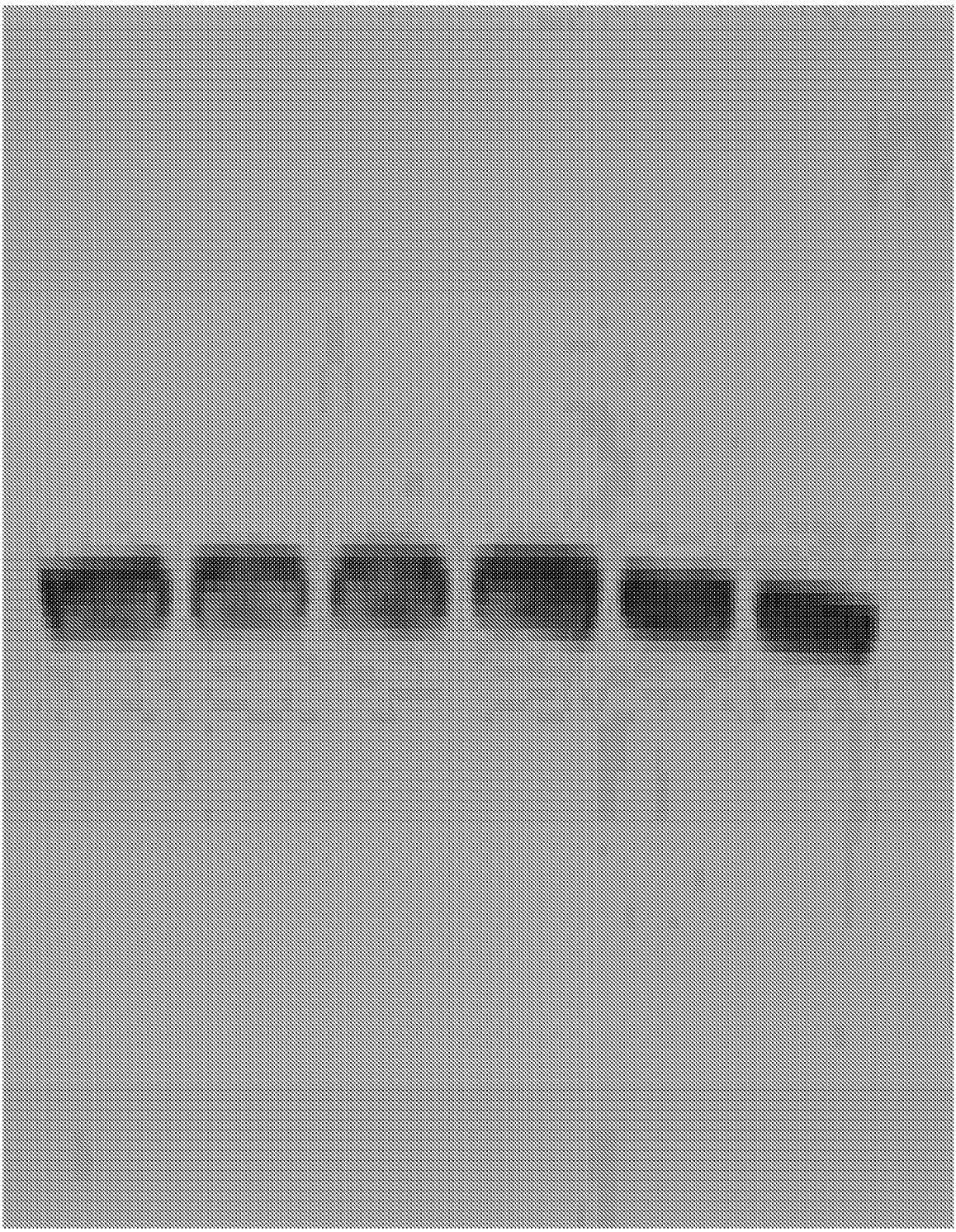

Figure 11

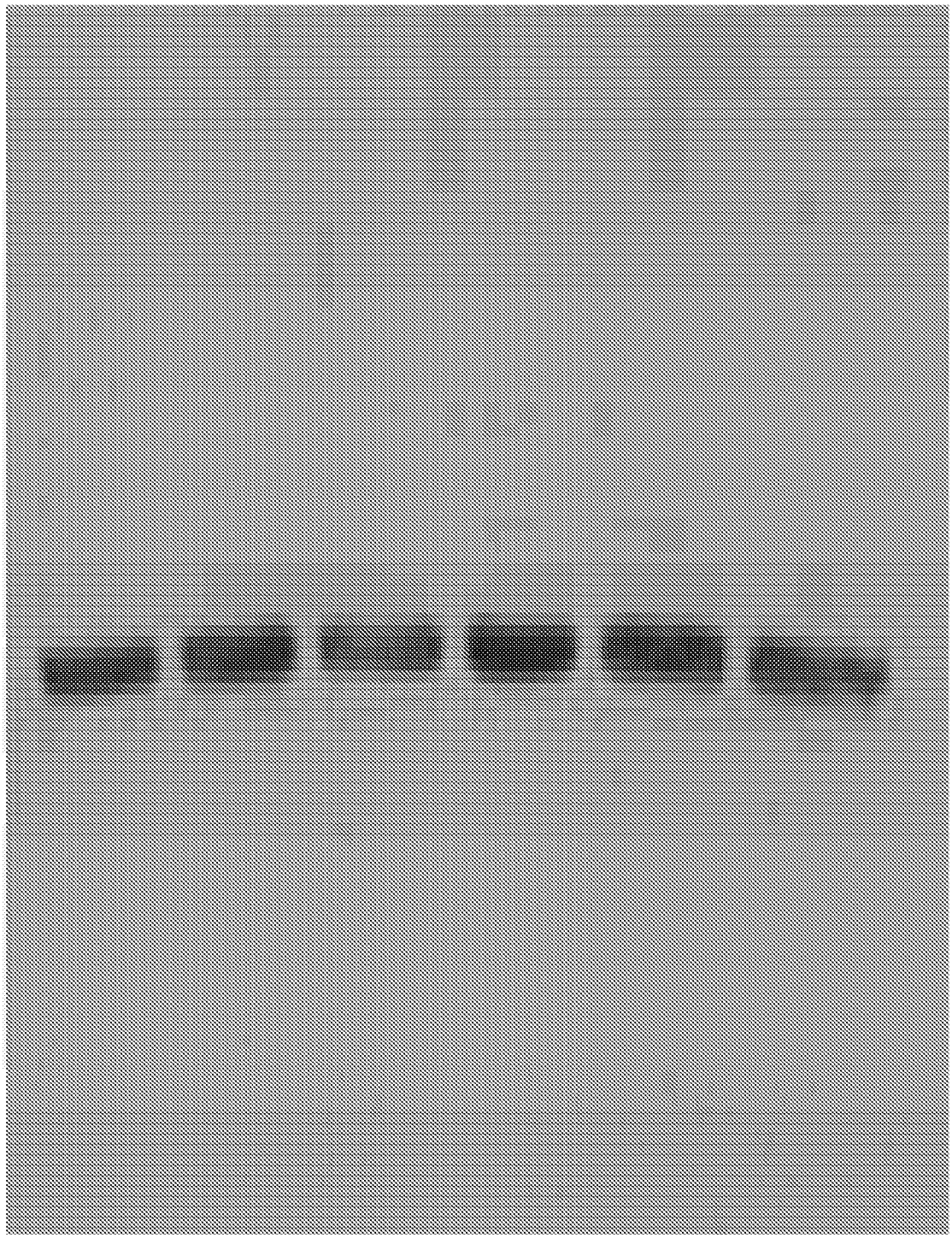

Figure 12

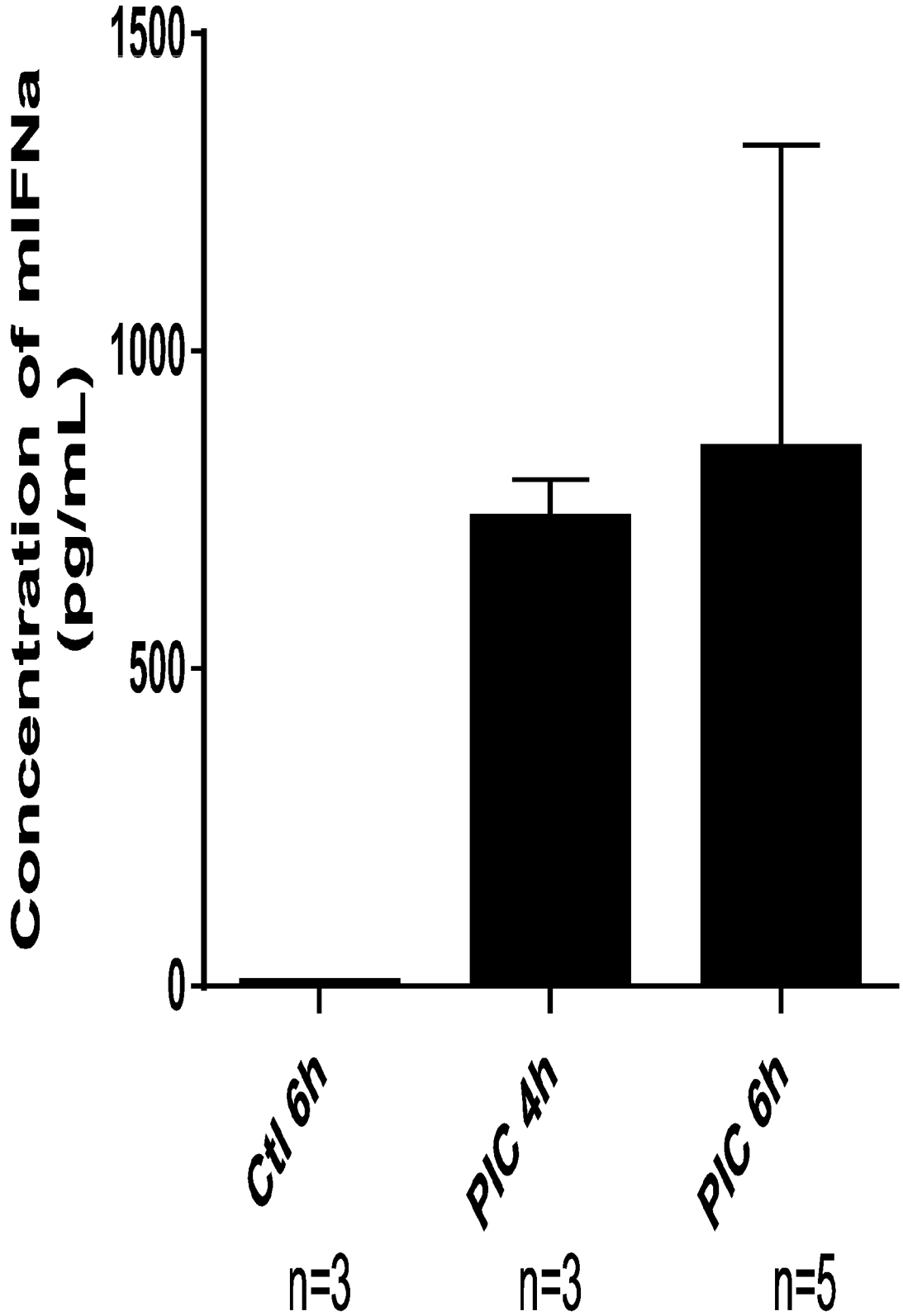
Concentration of mIFNa (pg/mL)
1500
1000
500
0
Ctl 6h
PIC 4h
PIC 6h
n=3
n=3
n=5

Figure 13

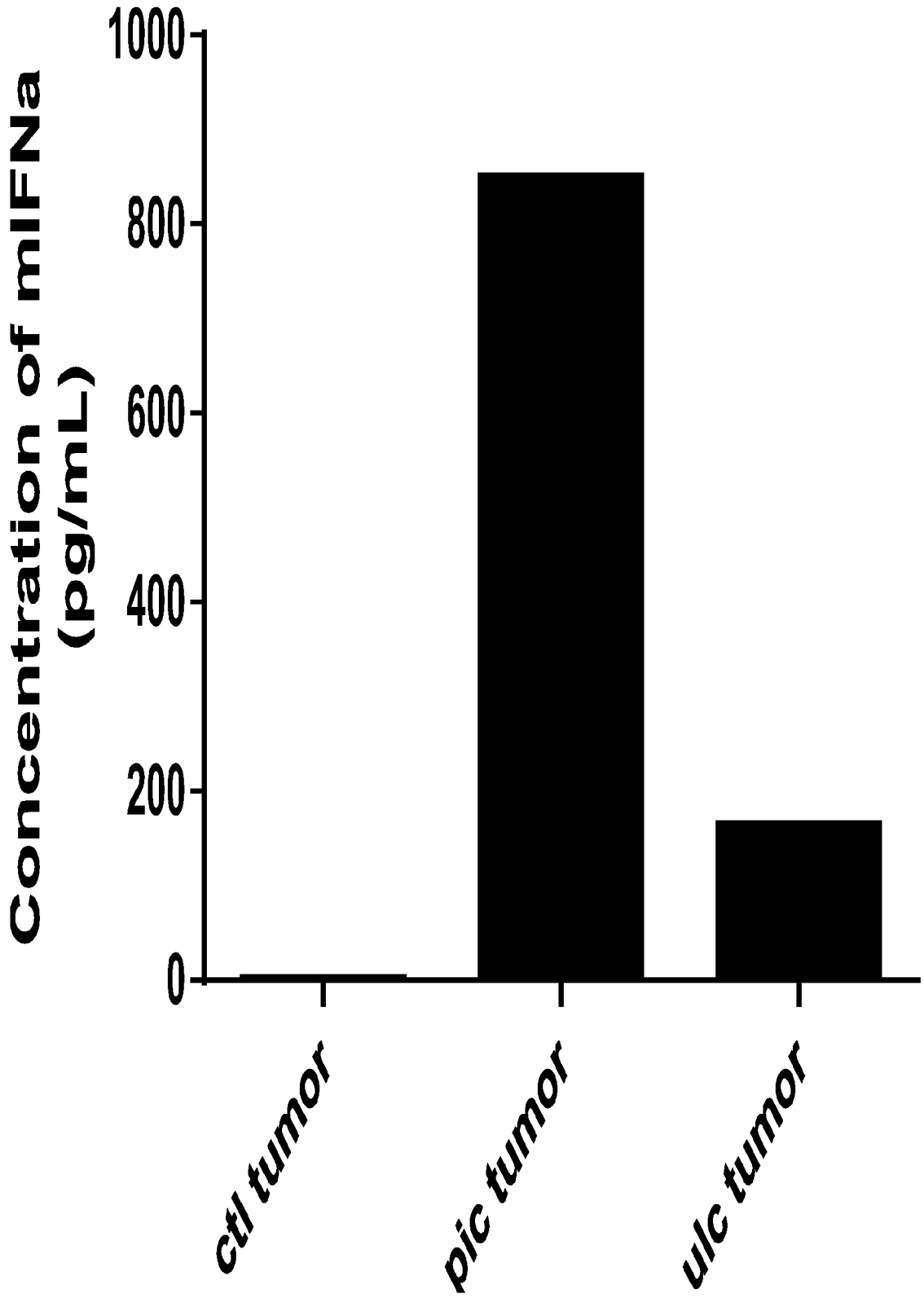
Concentration of mIFNa (pg/mL)
1000
800
600
400
200
0
ctl tumor
pic tumor
ulc tumor

Figure 14

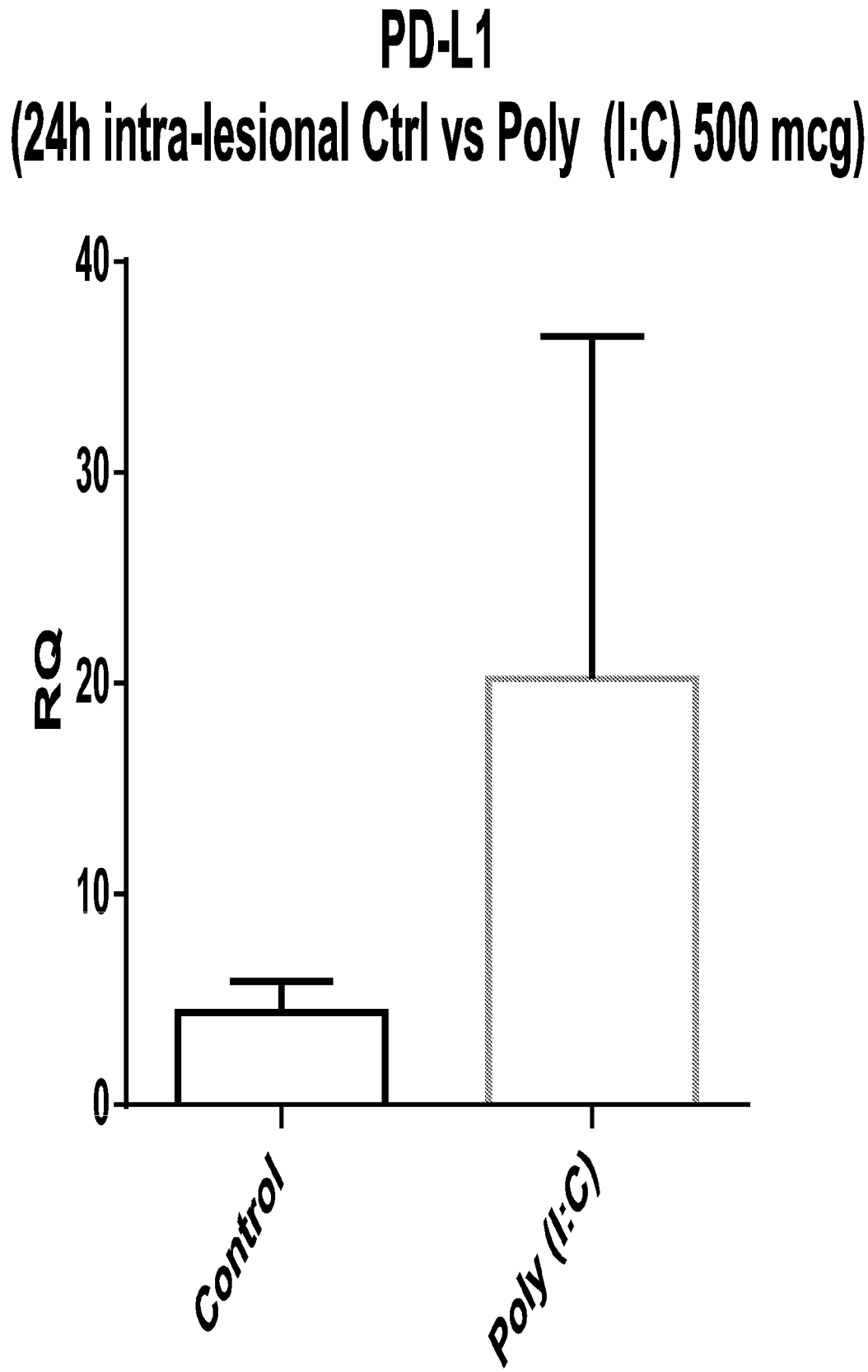
PD-L1
(24h intra-lesional Ctrl vs Poly (I:C) 500 mcg)
RQ
40
30
20
10
0
Control
Poly (I:C)

Figure 15

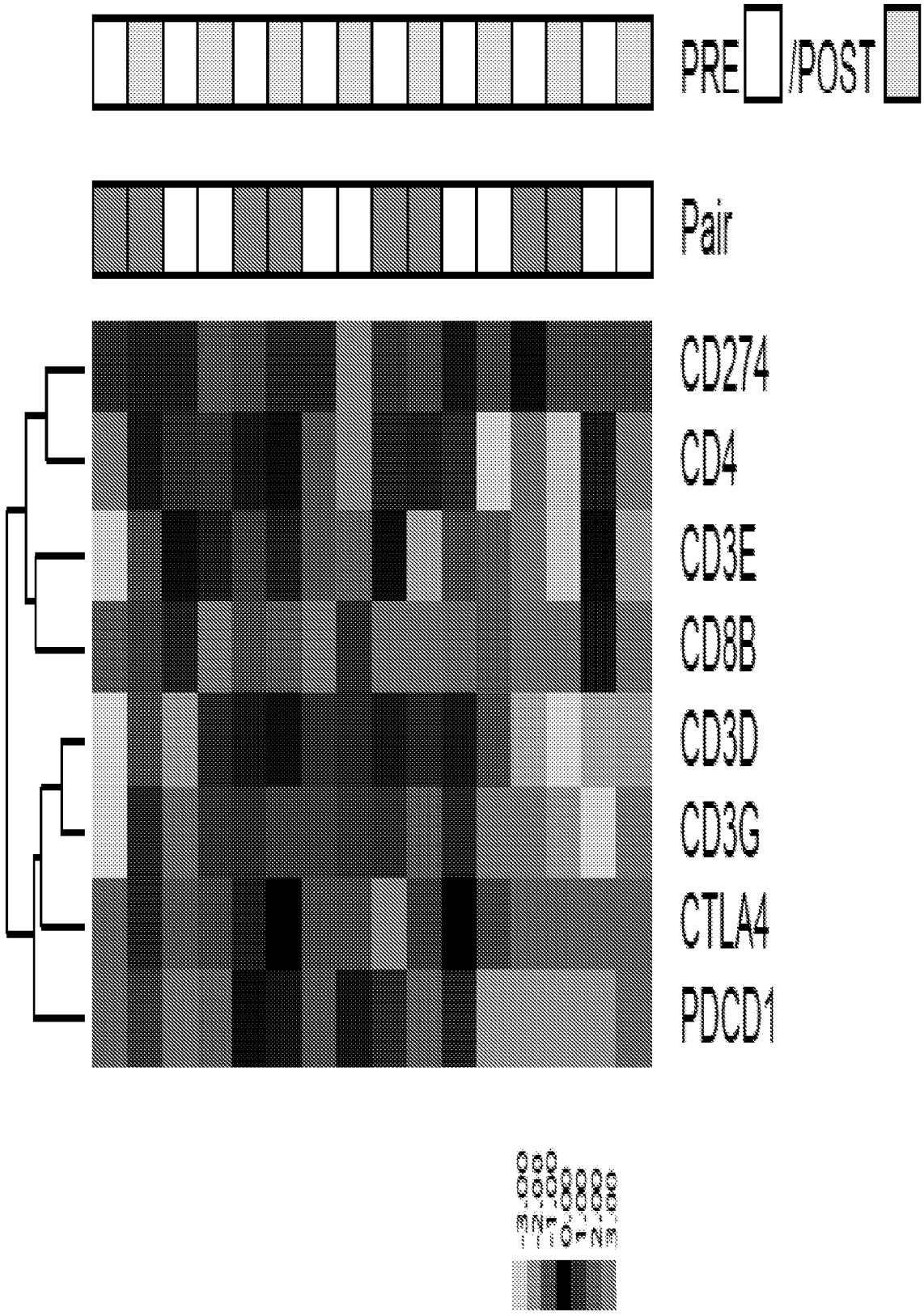

Figure 16

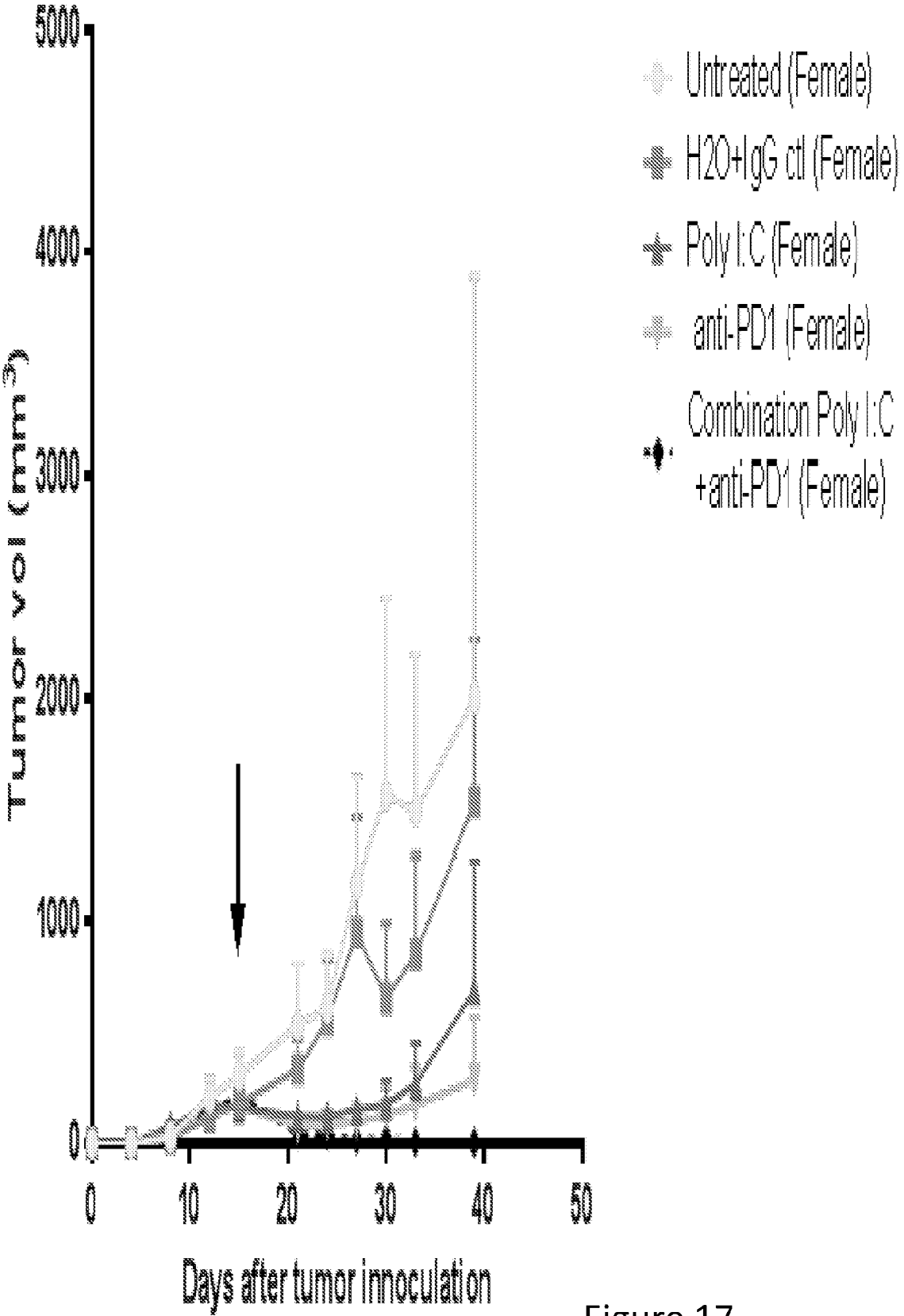
5000
4000
3000
2000
1000
0
Tumor volume (mm3)
0
10
20
30
40
50
Days after tumor innoculation
Untreated (Female)
H2O+IgG ctl (Female)
Poly I:C (Female)
anti-PD1 (Female)
Combination Poly I:C +anti-PD1 (Female)

Figure 17

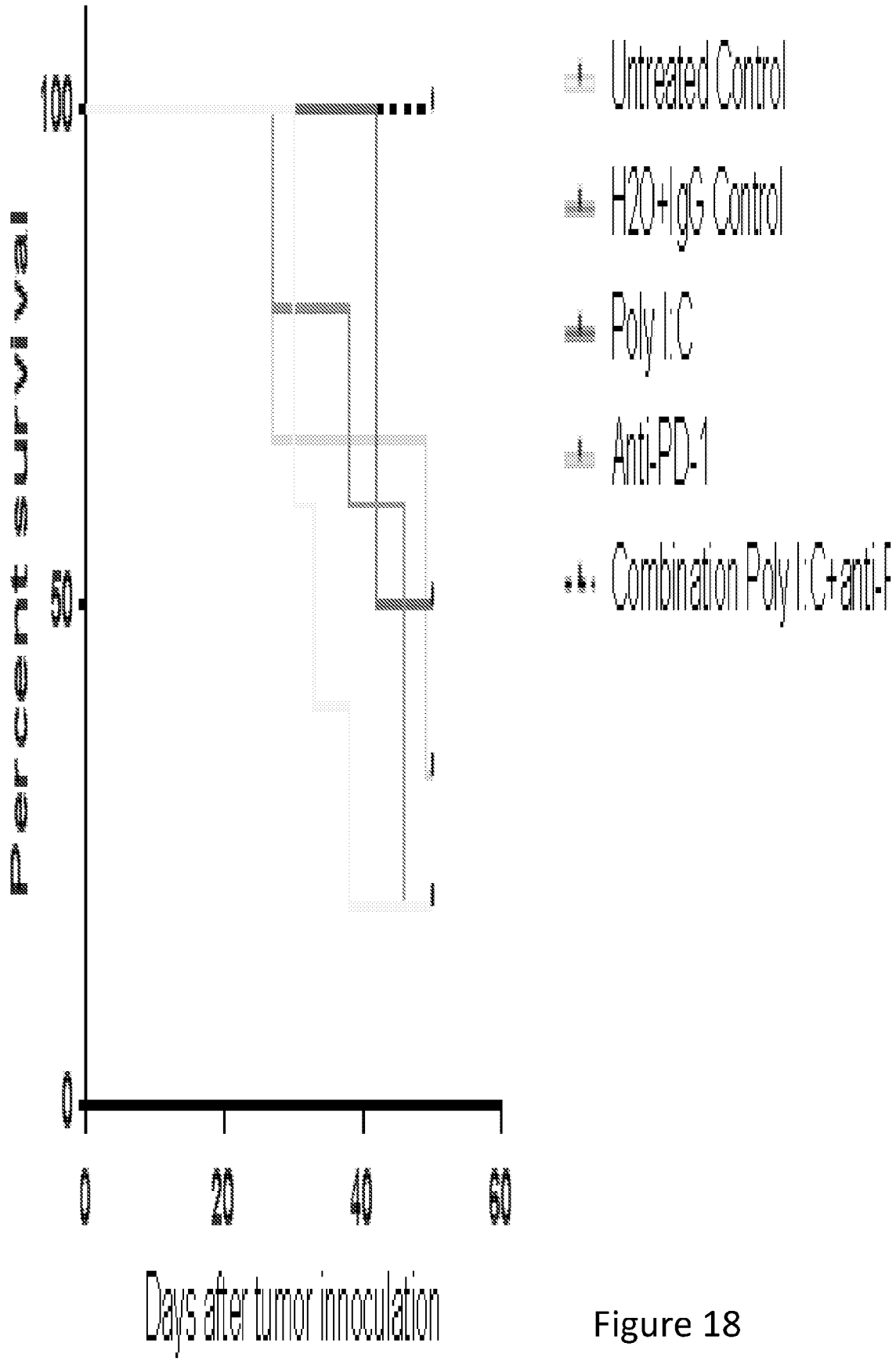
Percent survival
100
50
0
0
20
40
60
Days after tumor innoculation
Untreated Control
H20+IgG Control
Poly I:C
Anti-PD-1
Combination Poly I:C+anti-F

Figure 18

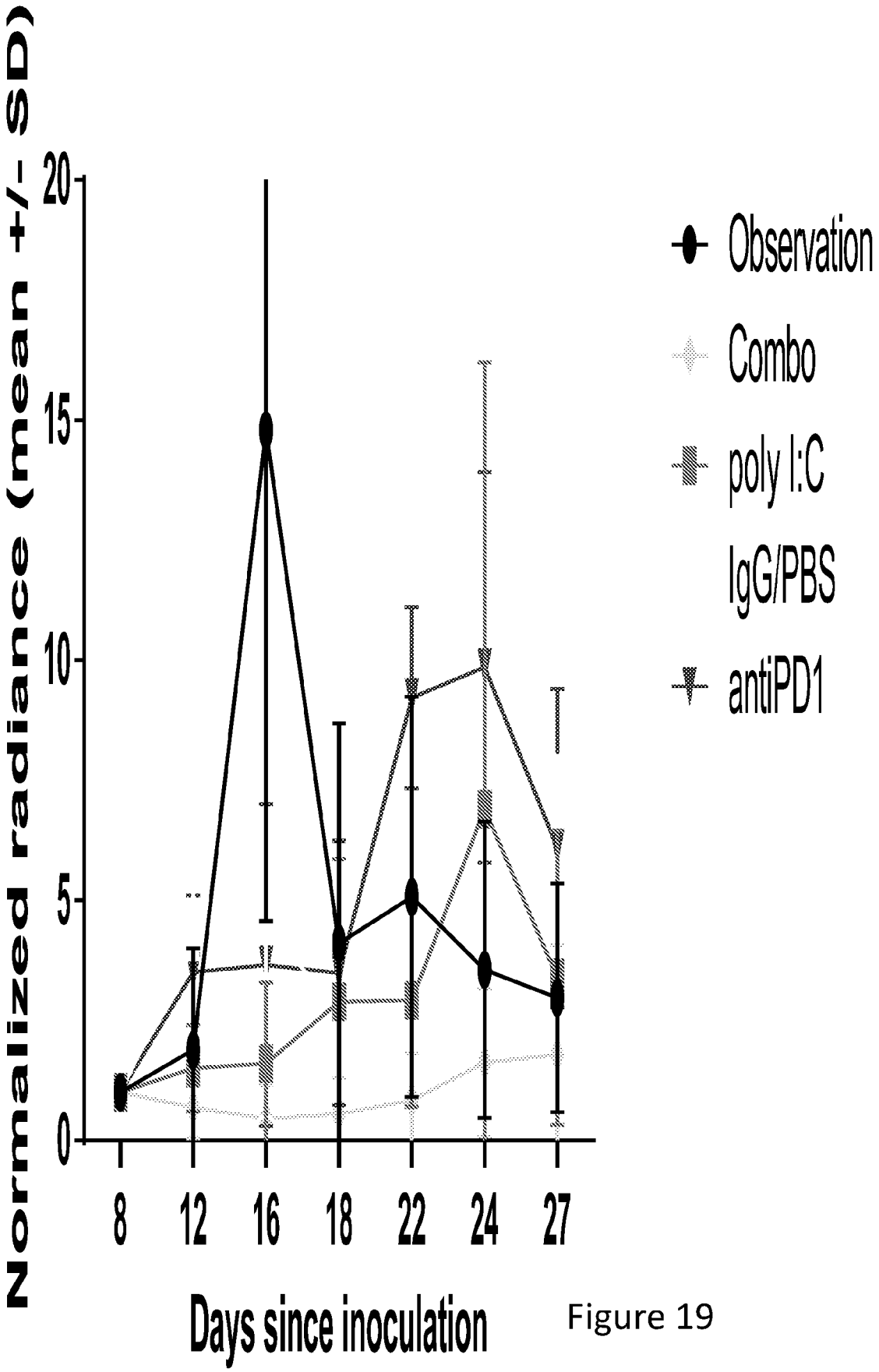
Normalized radiance (mean +/- SD)
20
15
10
5
0
8
12
16
18
22
24
27
Days since inoculation
Observation
Combo
poly I:C
IgG/PBS
antiPD1

Figure 19

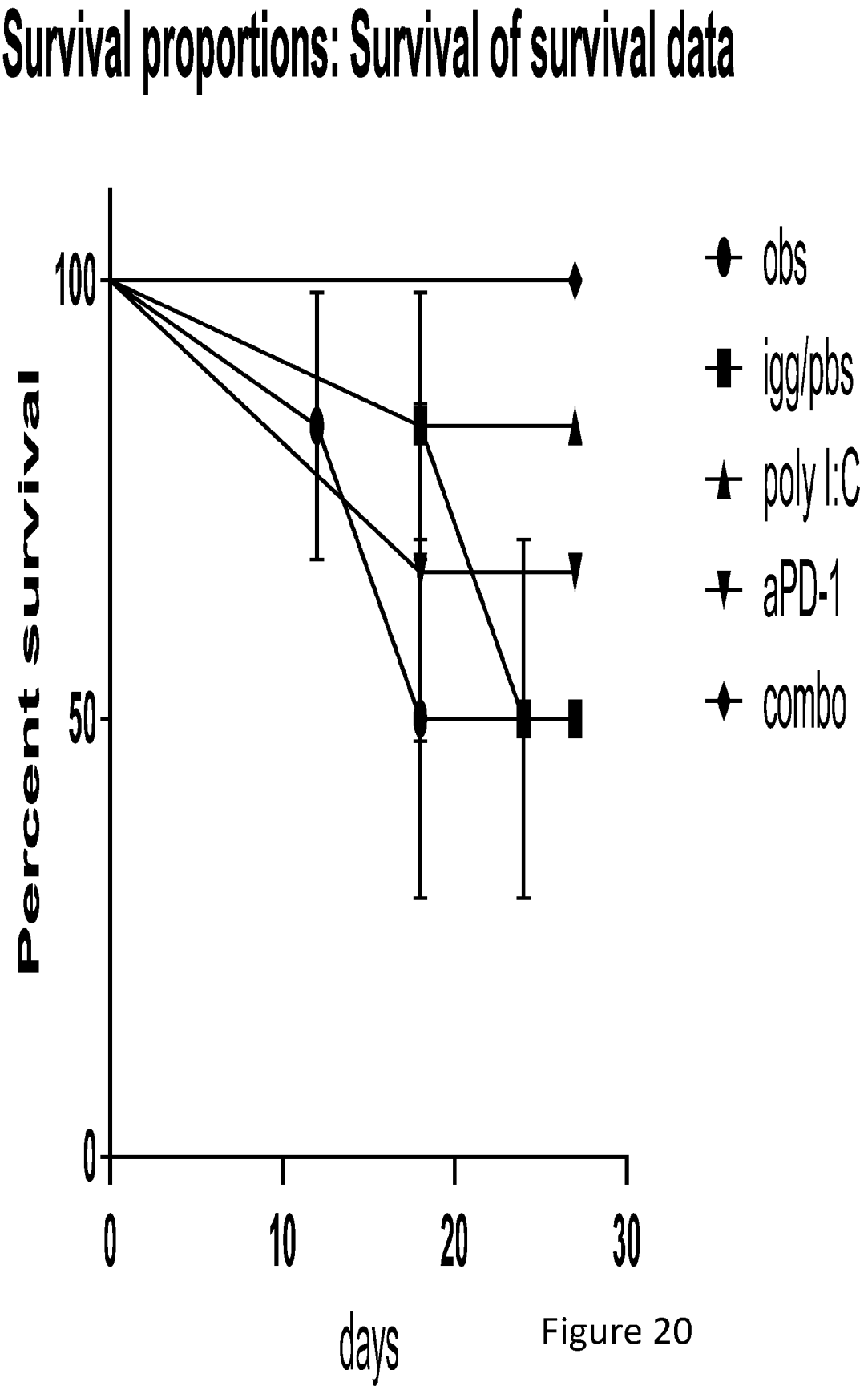
Survival proportions: Survival of survival data
Percent survival
100
50
0
0
10
20
30
days
obs
igg/pbs
poly I:C
aPD-1
combo

Figure 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/017568

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61P 35/00; A61P 35/04; A61K 35/76; A61K 35/761; A61K 39/00; A61K 39/0011 (2017.01)

CPC - A61K 2039/507; A61K 38/00; A61K 38/21; A61K 38/217; A61K 2039/505; A61K 2039/55522; C07K 16/2818; C07K 16/00; C07K 16/28; C07K 2317/24; C07K 2317/76 (2017.02)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/152.1; 424/183.1; 530/387.1; 530/388.15; 530/391.7 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X – Y	CA 2934073 A1 (THE BROAD INSTITUTE INC. et al) 25 June 2015 (25.06.2015) entire document	1-11, 15-19 --- 12-14
Y	BALD et al. "Immune Cell-Poor Melanomas Benefit from PD-1 Blockade after Targeted Type I IFN Activation," American Association for Cancer Research, 03 March 2014 (03.03.2014), Vol. 4, Iss. 6, Pgs. 674-687. entire document	12-14
A	ABIKO et al. "IFN-gamma from Lymphocytes Induces PD-L1 Expression and Promotes Progression of Ovarian Cancer," British Journal of Cancer, 31 March 2015 (31.03.2015), Vol. 112, No. 9, Pgs. 1501-1509. entire document	1-19
A	US 8,168,757 B2 (FINNEFROCK et al) 01 May 2012 (01.05.2012) entire document	1-19
A	OTT et al. "CTLA-4 and PD-1/PD-L1 Blockade: New Immunotherapeutic Modalities with Durable Clinical Benefit in Melanoma Patients," Clinical Cancer Research, 01 October 2013 (01.10.2013), Vol. 19, Iss. 19, Pgs. 5300-5309. entire document	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 March 2017	26 APR 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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